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PROGRAM REVIEW PROCEEDINGS OF: ENVIRONMENTAL EFFECTS OF ENERGY RELATED ACTIVITIES ON MARINE/ESTURINE ECOSYSTEMS

Interagency
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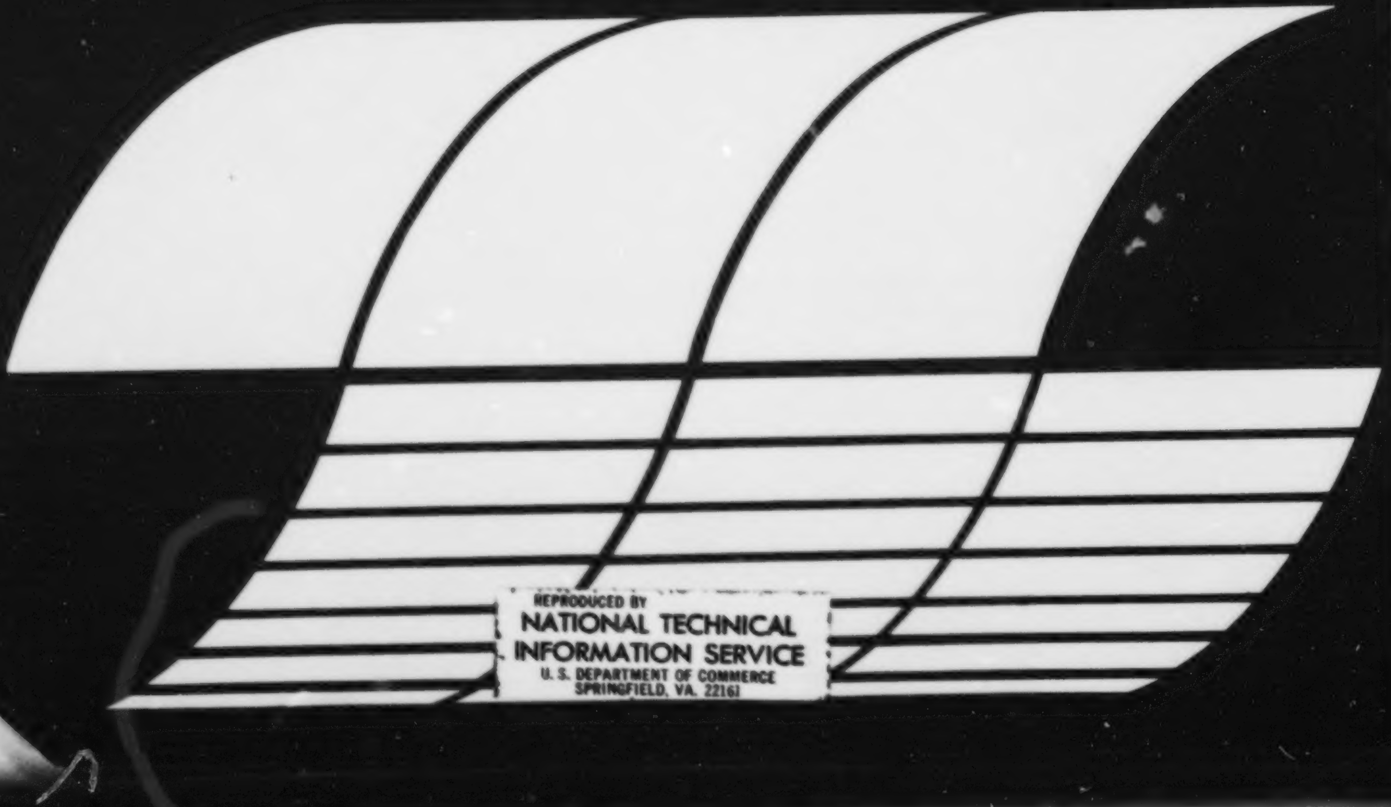
Program Report



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Fiscal year 1975 was the beginning of the Environmental Protection Agency's lead responsibility for the planning, coordination, and implementation of the Federal Interagency Energy/Environment R&D Program.

The "Report of the Interagency Working Group on Health and Environmental Effects of Energy Use," completed in Novemember 1974, recommended an integrated environmental sciences research program addressing needs in five major areas: Pollution Characterization and Monitoring, Environmental Transport Processes, Health Effects, Ecological Effects, and Integrated Assessment.

This report is the compilations of proceedings from the review session for the marine and esturine portion of the interagency program crosscutting the pollutant characterization and monitoring, environmental transport processes, and ecological subareas.

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PROGRAM REVIEW PROCEEDINGS OF: ENVIRONMENTAL EFFECTS OF ENERGY RELATED ACTIVITIES ON MARINE/ESTURINE ECOSYTEMS

compiled by

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FOREWORD

Although the Federal Government has been the major sponsor of energy research and development since the early 1950's, it has been only recently that energy/environment R&D has been of major public concern. Since fiscal year 1975, the Environmental Protection Agency has had the lead responsibility for the planning, coordination, and implementation of the Federal Interagency Energy/Environment R&D Program.

This Program, funded at approximately $100 million per year and participated in by seventeen Federal departments and agencies, is based on the recommendations of two interagency working groups commissioned by the Council on Environmental Quality and the Office of Management and Budget in 1974. The "Report of the Interagency Working Group on Health and Environmental Effects of Energy Use," completed in November 1974, recommended an integrated environmental sciences research program addressing needs in five major areas:

- Pollutant Characterization and Monitoring
- Environmental Transport Processes
- Health Effects
- Ecological Effects
- Integrated Assessment

The program is now in its fourth year. We are committed to keeping the Interagency Energy/Environment Program objective oriented. Accordingly, a series of program review and planning sessions have been scheduled for each subprogram area both to examine the science being conducted at the specific project level and also to draw on the knowledge and instincts of the participating scientists and program managers to forecast what the future research needs will be. It is hoped that in this manner we can adequately anticipate and provide the kind of information that will ensure sound environmental consideration in future energy policy and decision-making.

The following compilation represents the proceedings from the review session for the marine and estuarine portion of the interagency program -- cross-cutting the pollutant characterization and monitoring, environmental transport processes, and ecological effects subareas.

Clinton W. Hall

Clinton W. Hall, Director
Energy Coordination Staff
U.S. Environmental Protection
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SUBLETHAL EFFECTS OF PETROLEUM ON *MACOMA BALTHICA*

M.S. Stekoll, L.E. Clement, and D.G. Shaw*

The bivalve mollusc Macoma balthica has previously been proposed as an indicator of oil pollution (Shaw et al., 1976). This small clam was selected for study because of its wide distribution and abundance in Alaskan intertidal environments. Macoma balthica is a surface deposit feeder and suspension feeder, which would make it likely to concentrate both suspended and stranded oil that might occur following a spill (Brafield and Newell, 1961). Such concentrated oil in the clams might be further distributed throughout the ecosystem via migrating birds that feed on M. balthica.

Earlier experiments that subjected M. balthica to simulated oil slicks showed mortalities at an applied rate of 5.0 μg/cm^2 (Shaw et al., 1976; Shaw et al., 1977). Laboratory experiments indicated that initially buried clams would come to the surface when exposed to certain levels of Prudhoe Bay crude oil in the water (Feder et al., 1976). Macoma balthica, therefore, seems to respond to oil in its environment. It was the purpose of the present investigation to show that M. balthica is able to concentrate oil from an oil-sea water dispersion and to ascertain what effects the oil might have on membrane-bound enzymes and other physical and chemical parameters of the clams.

METHODS

Experimental clams were to be exposed to oil-sea water mixtures for 3 weeks, followed by a 4-week recovery period. For each treatment, oiled and control, 900 clams were used. Clams were divided into sampling lots and placed into 2 cm x 10 cm glass Petri dish bottoms, each containing 175g of silica sand and 15 clams. The Petri dishes were placed on an acrylic rack, which held 30 dishes in five levels. Two racks were used for each treatment. The racks were set in exposure chambers made of acrylic with dimensions of 24 cm x 66 cm x 30 cm.

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Each chamber was filled with about 33 to 35ℓ of sea water or oiled sea water and cooled by immersion in distilled water maintained at 10°C.

The clams used in this experiment were collected from the northern end of Resurrection Bay near Seward, Alaska, in September 1976. Clams were maintained in Instant Ocean aquaria for 2 months prior to the experiment. The clams were allowed to depurate in clean sea water at 5°C with aeration for 3 days just before exposure to the oil. All apparatus and sand that would come into contact with the clams were either heated at 500°C for 24 hr or extracted for several days in sea water to remove unwanted hydrocarbons.

The sea water used in this experiment was collected from Seward, Alaska, and stored in 55 gal stainless steel drums at 4° until used. The sea water in the exposure chambers was changed every other day. About 35ℓ of sea water were poured into 15 gal, all-glass aquaria and aerated through gas dispersion tubes for 2 to 3 hours. The control (no oil) was then siphoned into the previously drained exposure chamber.

One liter of sea water was removed from the water to be oiled. To this, about 200 μℓ of Prudhoe Bay crude oil was added. Addition of the oil was through a bent tip attached to a Gilson Pipetman adjustable pipet (0 to 200 μℓ). The oil was dispensed in small increments directly underneath a sonic probe (Biosonik) set at 15% of maximum, causing an oil-water dispersion. Droplet size was determined to be in the range of 1 to 5 μm. Enough of this oil-water dispersion was re-added to the sea water to give a final exposure concentration in the range of 1 to 2 μg of oil per milliliter of water. This oil-water mixture was siphoned into the exposure chamber.

Water changes were performed every other day starting on day 0 with the last change on day 18. On day 21, each set of racks was transferred to a separate 25 gal Instant Ocean tank filled with clean sea water and maintained at 8° to 10°C with aeration. The experiment was terminated on day 49.

Samples for hydrocarbon analysis were taken on days 0, 10, 21, 35, and 49. Ten dishes (150 clams) from each treatment were removed from the tanks and screened to remove sand. The clams were then depurated in clean sea water for 24 hr before shucking for hydrocarbon analysis. Sediment samples were collected on days 10 and 21 and frozen for later analysis. Water samples were collected at intervals (Exhibit 1) and extracted with hexane (see Appendix I for extraction and analysis procedures).

Samples for enzyme assays, glycogen, fresh and dry weights, and total lipid were collected on days 0, 7, 14, 21, 35, and 49. Three dishes were selected at random from each treatment chamber. Clams were screened from the sand and placed in beakers of clean sea water on ice. For enzyme assays, 10 clams from each Petri dish were shucked and homogenized in 10.0 mℓ of 0.25 M sucrose, 1.0 mM Na_2EDTA, pH 7.0 with a

Virtis '45' homogenizer set at top speed for two, 10 sec bursts. The homogenate was filtered through gauze and further homogenized in a Teflon-glass homogenizer at 1000 rpm for two strokes. Part of the homogenate was frozen for later assay for glucose-6-phosphatase, Mg-ATPase, and NA$^+$-K$^+$-ATPase. The rest of the homogenate was centrifuged at 1000 x g for 10 min, and the supernatant was respun at 17,300 x g for 20 min for a mitochondrial pellet fraction. The pellet was resuspended in 0.25 M sucrose, 1 mM EDTA, pH 7.0, and assayed for succinate dehydrogenase activity. (See Appendix II for enzyme assays.)

The remaining clams were allowed to depurate for 24 hr in clean sea water, then shucked into tared test tubes and frozen. After thawing and weighing, these clams were lyophilized to dryness, reweighed, and assayed for either glycogen or total lipids. (See Appendix III for assays.)

EXHIBIT 1: HYDROCARBON CONCENTRATIONS IN UNEXPOSED CLAMS, CLAMS EXPOSED TO PRUDHOE BAY CRUDE OIL, OILED, AND NON-OILED SEA WATER

Clams (μg hydrocarbons/g wet weight)

	Fraction 1*		Fraction 2*	
Day	Oiled	Control	Oiled	Control
0	18.62[†]		14.08[†]	
10	54.94 (4.8)	19.77 (3.4)	48.88 (4.8)	21.20 (5.0)
21	74.52 (0.6)	22.06 (5.0)	54.54 (5.2)	37.30 (3.4)
35	48.55 (7.0)	16.83 (1.0)	48.47 (9.0)[‡]	35.16[†]
49	38.96 (1.7)	18.77 (3.6)	46.82 (14.0)[‡]	42.55 (4.1)[‡]

Sea water (μg/mℓ)

Time	Oiled	Non-oiled
0 hr	0.64 (0.11) n = 4	0.014 (0.002) n = 2
after 48 hr	0.086 (0.02) n = 6	0.020 (0.004) n = 2

---

* Values are means (and standard deviations) of three determinations, except where noted, and quantified from the chromatograms in the region bounded by C_{14} and C_{32} normal alkanes, using an external standard. Fraction one contains saturated alkanes, and fraction two contains aromatic compounds, unsaturated hydrocarbons, and several other compounds of biological origin.

[†] n = 1.

[‡] n = 2.

RESULTS AND DISCUSSION

Part of our hypothesis in this experiment was that the oiled clams would show aberrations in their membrane-bound enzyme systems because of the lipid-lipid associations between hydrocarbons and membranes (Roubal, 1974). Since many membrane-bound enzymes need associated lipids to function effectively (White, et al., 1973), there was a possibility that the oil would disrupt this association (Manwell and Baker, 1967; Roubal, 1974). We also measured other parameters in the clams to determine possible general effects on proteins, lipids, glycogen, and fresh and dry weights of the clams.

The oiling treatment in this experiment appeared to have no deleterious effects on the clams. The enzyme assays showed no significant differences between the experimental group and the controls (Exhibit 2). Succinate dehydrogenase levels remained at 15 to 25 nmoles/min/mg protein. The Na^+-K^+-ATPase pump varied from 7 to 12 nmoles P_i/min/mg protein with no differences between the two groups. The Mg-ATPase levels for both groups rose initially, peaking at day 21, and then fell back again toward day 0 levels. Glucose-6-phosphatase remained fairly constant throughout the experiment.

Measurement of the other parameters also failed to show any significant differences between the two groups of clams (Exhibit 3). Both groups showed a slight decrease in dry/wet weight, which was a result of both a decrease in dry weight and an increase in wet weight. The protein/wet weight values rose initially, then dropped and leveled off for each group. There was a large variability in the glycogen values, making it difficult to observe any overall trend in glycogen content of the clams. The P_i/protein values dropped slightly during the experiment for both groups. There was also no difference between the control and experimental groups with respect to total extractable lipids.

There were two additional observations that indicated some difference between the control and the experimental groups. The first was the observation that the controls appeared to exhibit more siphon activity than the oiled clams. We did not measure this activity quantitatively, but whereas the control group extended their siphons in active suspension feeding behavior (Brafield and Newell, 1961), the oiled group displayed very little siphoning behavior. The second observation is that 19 control clams died (gaping shells) and only 2 oiled clams died during the first 21 days. The reason for this relatively high mortality in the controls is not known, but it may be related to oxygen availability as a function of siphoning activity.

When the clams were extracted for hydrocarbons, however, there were definite differences between the two groups. The oiled clams did indeed take up and concentrate hydrocarbons. Exhibits 4 and 5 indicate the qualitative differences between the clams on day 21. Exhibit 1

DISCLAIMER

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gives the values for hydrocarbons in the clams at different time periods. The oiled clams retained hydrocarbons throughout the end of the experiment.

Levels of hydrocarbons in the control and oiled waters are shown in Exhibit 1. Sediment from both the control and the oiled treatments contained very small amounts of hydrocarbons with no differences existing between the two.

EXHIBIT 2: ENZYME ASSAYS

Day	Oil	Succinate Dehydrogenase	Glucose-6-Phosphatase	MgATPase	Na^+-K^+-ATPase
0	-	22.9 ± 2.1	2.13 ± 0.25	32.9 ± 2.9	8.9 ± 0.6
0	+	21.4 ± 3.7	2.47 ± 0.24	30.6 ± 5.7	10.7 ± 3.5
7	-	17.0 ± 2.7	1.82 ± 0.32	41.2 ± 2.9	8.0 ± 1.7
7	+	21.7 ± 2.3	1.65 ± 0.46	41.6 ± 6.9	7.1 ± 4.1
14	-	17.3 ± 0.9	1.78 ± 0.38	39.6 ± 2.6	10.1 ± 1.4
14	+	15.6 ± 1.7	2.11 ± 0.17	39.7 ± 4.7	9.0 ± 0.2
21	-	24.0 ± 3.8	2.19 ± 0.55	52.3 ± 6.9	8.7 ± 1.8
21	+	20.0 ± 2.7	1.98 ± 0.34	47.1 ± 4.5	9.0 ± 1.9
35	-	22.8 ± 2.4	1.82 ± 0.19	44.5 ± 2.4	9.2 ± 0.4
35	+	26.7 ± 3.8	1.73 ± 0.06	47.7 ± 5.8	8.9 ± 2.7
49	-	19.9 ± 1.2	1.94 ± 0.13	38.9 ± 3.2	8.1 ± 3.0
49	+	23.2 ± 2.4	1.54 ± 0.32	39.4 ± 2.7	12.6 ± 0.9

Note: Assays were performed in triplicate on three sets of ten clams each (N=3). Rates are expressed as nanomoles/min/mg protein ± S.D.

EXHIBIT 3: SOME PARAMETERS MEASURED ON MACOMA BALTHICA DURING 49-DAY OILING EXPERIMENT

Day	Oil	$\frac{\text{Pi}^{1*}}{\text{Protein}}$ $\frac{\text{(nmoles)}}{\text{mg}}$	$\frac{\text{Protein}^{*}}{\text{Wet Weight}}$ $(\frac{\text{mg}}{\text{g}})$	$\frac{\text{Dry Weight}}{\text{Wet Weight}}$ (n)	(%)	$\frac{\text{Glycogen}}{\text{Wet Weight}}$ (n)	$(\frac{\text{mg}}{\text{g}})$	$\frac{\text{Lipid}}{\text{Wet Weight}}$ (n)	$(\frac{\text{mg}}{\text{g}})$
0	-	235 ± 28	67.5 ± 8.0	(15)	20.6 ± 1.1	(8)	10.0 ± 4.6	(6)	16.7 ± 3.0
0	+	256 ± 28	68.9 ± 5.8	(15)	20.7 ± 0.6	(8)	7.0 ± 3.9	(6)	18.5 ± 2.3
7	-	212 ± 4	82.6 ± 1.8	(14)	20.2 ± 0.9	(7)	5.2 ± 4.2	(7)	18.1 ± 4.0
7	+	222 ± 14	76.8 ± 7.1	(15)	20.8 ± 1.0	(8)	8.4 ± 2.3	(7)	14.5 ± 2.6
14	-	212 ± 8	78.7 ± 1.7	(14)	21.0 ± 1.7	(7)	12.5 ± 2.1	(7)	17.4 ± 4.0
14	+	208 ± 10	75.5 ± 3.4	(15)	20.4 ± 0.8	(8)	9.5 ± 5.5	(7)	16.6 ± 3.3
21[†]	-	221 ± 18	64.5 ± 2.9	(12)	20.2 ± 1.1	(6)	7.1 ± 4.6	(6)	17.2 ± 4.1
21	+	210 ± 4	66.0 ± 3.8	(13)	20.5 ± 1.2	(6)	6.8 ± 3.5	(7)	19.8 ± 4.2
35	-	219 ± 16	69.1 ± 0.7	(9)	18.9 ± 1.3	(5)	6.3 ± 5.0	(4)	18.9 ± 4.9
35	+	221 ± 7	70.8 ± 2.9	(14)	18.8 ± 1.2	(7)	7.4 ± 4.6	(7)	17.8 ± 2.9
49	-	193 ± 6	68.7 ± 1.8	(14)	15.3 ± 1.1	(7)	3.0 ± 1.4	(7)	14.8 ± 1.7
49	+	193 ± 10	67.6 ± 1.4	(14)	15.3 ± 0.7	(7)	3.9 ± 2.9	(7)	15.2 ± 3.4

[*] From three sets of 10 clams each ± S.D.

[†] Oiling regime stopped at day 20.

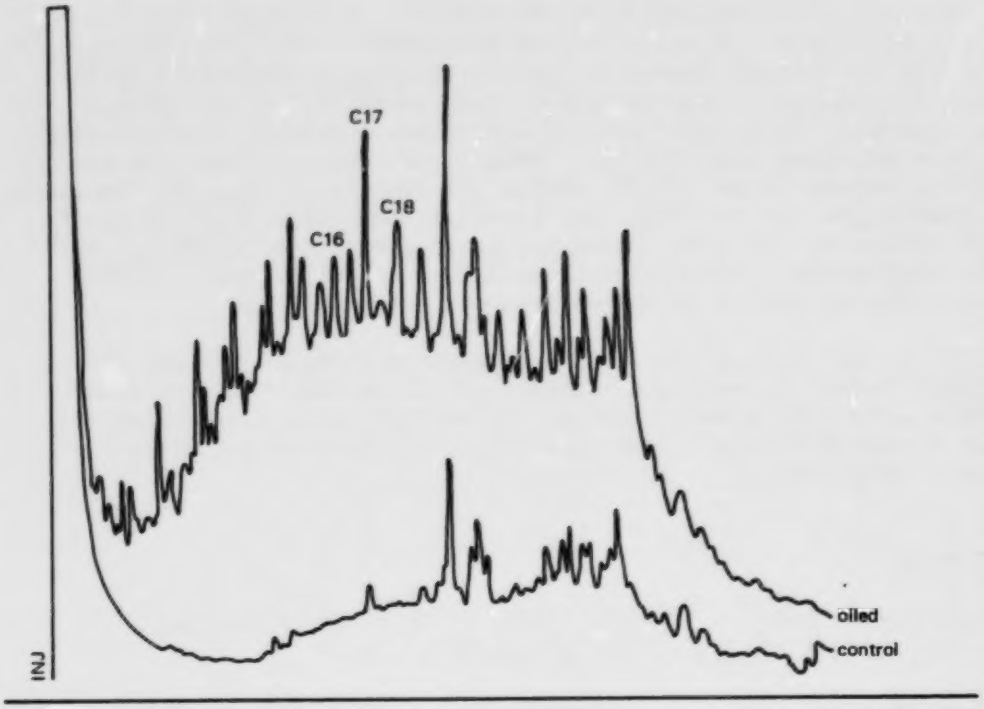

EXHIBIT 4: GAS CHROMATOGRAMS OF ALKANE FRACTIONS FROM DAY 21 CLAMS

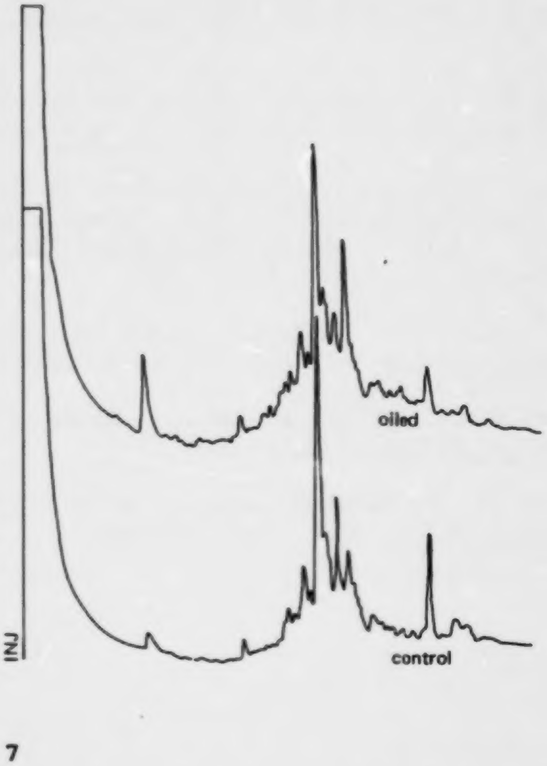

EXHIBIT 5: GAS CHROMATOGRAMS OF AROMATIC FRACTIONS FROM DAY 21 CLAMS

Although the clams concentrated portions of Prudhoe Bay crude oil in this experiment, we could find no measurable differences between the oiled and the control clams with respect to enzyme activities or other measured parameters. The relatively high mortality of the control clams, however, indicates that the experimental set-up induced a stress on the clams apart from the oil. This stress may have been the lack of aeration between water changes and/or the absence of food for the clams. The oiled clams did not show this mortality perhaps because of their lower filtration activity, which may be an indication of lowered metabolic functioning. A decrease in metabolic activity would certainly place a smaller demand on the environment for survival.

It is our feeling that longer exposure periods to oil are needed in order to see any sublethal effects of oil on the clams. A flow-through system would reduce the stress and mortalities caused by the water change procedure. Such a system is currently being developed in this laboratory.

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APPENDIX I: ASSAYS FOR HYDROCARBONS IN CLAMS, WATER AND SEDIMENT

General

All solvents used were redistilled in glass and then checked for purity by gas chromatography.

All glassware was washed with soapy water, rinsed in distilled water, and rinsed with organic solvents prior to use.

Samples were concentrated on a rotary evaporator to approximately 2 mℓ. They were then concentrated to final volume under a stream of dry nitrogen.

Gas chromatography (GC) was performed using a Hewlett Packard model 5710A gas chromatograph with flame ionization detectors. GC output was recorded on both an HP model 3380A integrator and a Sargent model SR recorder with a disc integrator. Sample components were separated using a 12 ft x 1/8 in. column packed with 3% OV101 on 100/120 chromosorb W. Oven temperature was programmed from 80°C to 280°C at 8°C/min. Carrier gas was nitrogen at 50 mℓ/min. Individual peaks were quantified using the electronic integrator, the total chromatogram by disc integration. Both integrators were calibrated using an n-alkane standard.

Clams

Each group of approximately 50 clams was thawed and then removed from their shells to a preweighed 50 mℓ centrifuge tube. Wet weight was determined, 5 mℓ of 8N NaOH was added, and the tube sealed with a teflon-lined cap. The tubes were then placed in a 90°C water bath for 3 hr, and were shaken every hour. After digestion, 15 mℓ of benzene were added, the tubes shaken vigorously and centrifuged at 2000 rpm for 10 min. The benzene phase was pipetted off, and the extraction procedure repeated twice using 10 mℓ benzene and 5 mℓ water, saturated with NaCl. The three extracts were combined and dried over Na_2SO_4. The benzene extract was concentrated to approximately 0.5 mℓ and placed on a 0.9 cm x 22 cm column packed with 5 g of 6% deactivated alumina over 5 g of 5% deactivated silica gel. The column was eluted with 20 mℓ of hexane (fraction 1) and then with 20 mℓ of benzene (fraction 2). Fraction 1 was concentrated to approximately 0.2 mℓ, fraction 2 to approximately 0.5 mℓ, and a 5 mℓ aliquot of each was gas chromatographed.

Water

Hydrocarbon content of freshly oiled sea water was determined on days 1, 5, 7, and 9. The amount remaining in this sea water after the 48 hr exposure period was determined on days 3, 5, 7, 9, 11, and 19. The hydrocarbon content of unoiled sea water was determined before exposure on days 3 and 17, and after 48 hr/exposure on days 3 and 19. Sample sizes were 500 mℓ of freshly oiled sea water, 1000 mℓ for oiled sea water after 48 hr, and 1000 mℓ for all unoiled sea water. The sample was extracted three times with a 5% sample volume of hexane. The hexane extracts were combined and dried over NA_2SO_4. The hexane extract was concentrated to approximately 0.5 mℓ, and a 5 mℓ aliquot gas chromatographed.

Sediment

A 100g sample of sand was collected after screening from the oiled chamber and from the control chamber on days 10 and 21. Each sample was extracted with 250 mℓ of refluxing methanol with stirring for 24 hr. The hydrocarbons were then partitioned into hexane; the hexane extract was dried over Na_2SO_4 and concentrated to approximately 1 mℓ. A 5 mℓ aliquot was gas chromatographed.

APPENDIX II: PROCEDURES FOR ENZYME ASSAYS

Glucose-6-Phosphatase (Baginski *et al*., 1974; Hübscher and West, 1965)

Assay Mix

			Treatments	A	B	C	D
1.	Sucrose/EDTA/KF*	0.10 ml		+	+	+	+
2.	Cacodylate buffer	0.10 ml		+	+	+	+
3.	Test solution	0.10 ml		(a)	(b)	(c)	(d)
	keep on ice - then at t=0 add						
4.	G-6-P	0.10 ml		+	+	+	+

Mix (vortex) and incubate 30 min at 30°C. Stop each reaction with 2.0 mℓ of cold TCA/ascorbate (10%, 2%) (W/V). Put on ice for 2-3 min, then centrifuge for 3 min to precipitate protein. Remove 1.0 mℓ of the supernatant to assay for phosphate.

* To make solutions:

1. Sucrose/EDTA/KF
   16 mM Na_4EDTA
   8 mM KF
   250 mM sucrose - adjust pH to 7.0 with HCl.
2. Cacodylate buffer (0.10 M)
   0.10 M cacodylic acid, pH 6.5

dissolve 0.138 g cacodylic acid in H_2O. Adjust pH to 6.5 with NaOH. Dilute to 10 mℓ. Keeps 1 week.

3. G-6-P (0.10 M)

   Dissolve 0.282 g Na G-6-P in 10 mℓ H_2O. Store cold. Keeps 1 week.

4. Test solutions:

   (a) Blank: 0.100 mℓ of 0.25 M sucrose/1 mM/EDTA

   (b) PI STD: 0.020 mℓ of 10 µmole/mℓ K_2HPO_4 + 0.080 mℓ of sucrose/EDTA (0.25 M/1mM).

   (c) Control: 0.100 mℓ of crude homogenate added after TCA/ascorbate

   (d) Crude homogenate: 0.10 mℓ of the crude homogenate.

KF and EDTA are included to inhibit alkaline and acid phosphatous (Hübscher, 1965).

Na^+-K^+-ATPase Assay (Jorgensen, 1974; Skou, 1957; Dunson and Dunson, 1975)

		Treatments (in triplicate)				
Add in order	mℓ	A	B	C	D	E
1. H_2O	0.430	+	+	+	+	+
2. 0.5 M imidazole HCl pH 7.5	0.100	+	+	+	+	+
3. 0.1 M $MgCl_2$	0.040	+	+	+	+	+
4. 10 mM Na_2EDTA	0.050	+	+	+	+	+
5. 1 M NaCl	0.020	+	+	+	+	-
6. 1 M KCl	0.020	+	+	+	+	-
7. 10 mM ouabain	0.200	-	-	-	-	+
8. Test solution	0.100	(a)	(b)	(c)	(d)	(e)
9. 75 mM ATP	0.040	+	+	+	+	+
Total Volume	1.000					

note: (-)=H_2O in place of solution.

Tubes are kept on ice until ATP is added, at which time tubes are vortexed and set in 20°C water bath for 10 min.
Test solutions

(a) Blank 0.100 mℓ of sucrose/EDTA (0.25 1mM)

(b) Pi STD 0.020 mℓ of 10 μmoles/mℓ phosphate K_2HPO_4
0.080 mℓ of sucrose/EDTA (0.25 M/1mM)

(c) Control - 0.100 mℓ of crude homogenate (diluted with sucrose/ EDTA if necessary) added after reaction is stopped with TCA/ ascorbate.

(d) Total ATPase - 0.100 mℓ of crude homogenate

(e) Mg-ATPase - 0.100 mℓ of crude homogenate

Stop reaction with 2.0 mℓ of cold ascorbate/TCA (2.5%/12.5%). (W/V). Vortex. Set on ice 5-10 min. Centrifuge precipitated protein 3-5 min on clinic-1 centrifuge (∿2400 rpm). Remove 1.0 mℓ of supernatant for phosphate analysis. Na^+-K^+-ATPase is difference between total ATPase and MgATPase.

Succinate Dehydrogenase (Singer, 1974; Bonner, 1955)

Preparation of Reagents:

1. KCN 0.1 M; to 70 mℓ of cold H_2O add 0.6512 gm KCN. Then add 8.5 mℓ of cold 1 N HCL to neutralize KCN. Keep stoppered for 10 min to readsorb KCN fumes. Then make up to the mark with cold H_2O. Store cold. Reagent is good for 2 days.

2. $K_3FE(CN)_6$ 0.01 M; to 100 mℓ H_2O add 0.3293 gm of potassium Ferricynide (Sigma 99%). Keep in a dark bottle protected from light. to

3. Na succinate 0.20 M; adjust to pH 7.5 at room temperature.

4. KPO_4 buffer, 0.30 M, pH 7.5 at room temperature.

Assay Procedure:

Into 3.0 mℓ cuvettes add in order:

			Final Conc.
1.	KCN 0.1 M	0.300 mℓ	0.01 M
2.	$K_3Fe(CN)_6$ 0.01 M	0.400 mℓ	0.33 K10^{-3} M
3.	Na succinate 0.2 M	0.200 mℓ	1.33×10^{-2} M
4.	KPO_4 buffer pH 7.5	1.000 mℓ	0.100 M
5.	H_2O	0.850 mℓ	-
6.	Diluted mitochondrial prep.	0.250 mℓ	-
		3.000 mℓ	

Add mitochondrial prep after all reagents have come to room temperature (20°C). Place cuvette in spectrophotometer at 20°C and measure ΔOD at 420 nm.

- Measure temperature at end of the run.
- A blank (control) is run substituting water for Na-succinate. This is subtracted from the (+) succinate cuvette.
- ΔOD should decrease at about $1-10 \times 10^{-3}$ OD/min.

Note: One mole of succinate reduces 2 moles of $Fe(Cn)_6$.

ϵ_{mM} for $Fe(CN)_6 = 1.0$ at 420 nm.

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APPENDIX III: ASSAYS FOR PHOSPHATE, GLYCOGEN, AND TOTAL LIPIDS

Phosphate Assay (Baginski *et al.*, 1974)

To 1.0 mℓ of incubation supernatant, which contains $\sim$1.67% ascorbate and 8-9% TCA add:

0.50 mℓ 1% (W/V) ammonium molybdate in H_2O. (Note: H_2O must be very pure.)

Mix contents immediately (vortex). Wait exactly 5.0 min (or some other exact time between 3-10 min). Then add:

1.00 mℓ of sodium arsenite/sodium citrate (each 2% W/V) in 2% acetic acid (V/V).

Mix contents immediately and let stand 15 min or longer. Read absorbance at 700 nm vs blank containing 1.0 mℓ H_2O + Pi reagents (or 1.0 mℓ of diluted ascorbate/TCA).

Color is stable for several hr.

Assay for Glycogen (Seifter, 1950)

Select 5-10 clams. Set in clean sea water for 24 hr at 4°C to attempt to eliminate sand from the clams. Add 1.0 mℓ of 30% KOH to dried clams in test tubes. Digest clams in a 95°C bath with marble caps. Incubate 40-60 min until all of clam is digested.

Cool digest and add 1.25 mℓ of 95% EtOH. Vortex to mix. Heat to gentle boil on water bath ($\sim$95°C), using marbles to cap the tubes. Cool. Centrifuge at 2300 rpm for 15 min (International model K). Pour off supernatant. Wash pellet by resuspending in 1.0 mℓ H_2O + 1.25 mℓ EtOH. Heat, cool, and centrifuge again. Pour off supernatant. Allow tubes to drain inverted on paper towels.

Resuspend pellet in 5.0 mℓ of H_2O. Heat to dissolve glycogen. Cool to room temperature. Remove 0.5 mℓ to assay for carbohydrate by the anthrone method. May store frozen, if necessary.

Express results as mg glycogen/gm fresh wet.

$$\text{mg glycogen in aliquote} = \frac{100 \times A_{620} \text{ of sample}}{1.11 \times A_{620} \text{ of 100 mg std}}$$

Anthrone Assay (Spiro, 1966)

Reagents:

280 mℓ H_2O
+720 mℓ conc H_2SO_4 (reagent grade).
While still warm, add:

500 mg anthrone
10.0 g thiourea

Mix to dissolve. Cool and store at 4°C. Age 7 hr. Reagent will keep about 2 weeks.

Procedure:

Use any test tube >10 mℓ capacity
range 20-200 μg hexose in 1.0 mℓ H_2O
1.0 mℓ sample on ice
5.0 mℓ cold anthrone reagent

Vortex to mix. Keep on ice until all tubes are ready. Cap with marbles. Place in boiling H_2O for exactly 10 min. Remove and cool in H_2O bath to room temperature. Let stand 20 min. Read absorbances at 620 nm.

Lipid Extraction (Radin, 1969)

Add 100 mℓ of distilled H_2O to lyophilized clam in 14 x 100 mm test tube. Allow to rehydrate for 20 min. Grind up clam with clean glass rod. Rinse rod 3 times with 0.5 mℓ benzene-MeoH (2:1 v/v). Add rinses to tubes containing clams. Vortex. Sonicate with small needle probe for 5 seconds at 'min' and 5 secs at 50%. Cap tubes with marbles and incubate for 20 min at 50-55°C. Centrifuge and place supernatant into clean large test tubes. Wash residue two times with 2.0 mℓ and then 1.0 mℓ of benzene:MeoH, 2.1 (v/v). Add washings to supernatant.

Add 1.0 mℓ of 0.75% NaCl to extract water soluble lipids. Vortex. Centrifuge. Remove upper layer; place in tared vial (if there is an emulsion or more than 2 layers, add more NaCl). Re-extract water layer two times with 1.0 mℓ benzene. Combine all benzene fractions in the tared vial.

Evaporate contents of vial under N_2 stream. When dry, lyophilize to remove water residue. Re-weigh dried vial to obtain weight of total lipids in clam.

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DISCUSSION

Dr. McErlan: If a more natural system was established that allowed the sediment to be contaminated around the animal, an entirely different result might be obtained. Generally, the Macoma uses two siphons, which pick something off the interface of the water column at the bottom. This method is its primary means of obtaining food. It is much quicker than straining water.

Dr. Stekoll: The Macoma feeds both ways. They stick their siphons out as if it is in suspension and they deposit feed. However, we are mainly concerned with the effects of the oil -- not with their feeding mechanisms. Our experiment did not demonstrate any difference between the oiled and controlled clams.

Dr. Dieter: Did the oiled sea water contain about 0.5 mg/gm of alkanes, and, did your clams, subtracting the values from the controls, have from 20 to 50 mg/gm of alkanes?

Mr. Stekoll: They concentrate approximately 80 times.

Dr. Dieter: In analyzing petroleum hydrocarbons in the clams, what is your percent recovery?

Mr. Stekoll: 70 to 90. We put in an internal standard of C-14, C-21, C-30, to get our recovery back and correct for that.

Dr. Rogerson: Your recoveries from the aromatic fractions appear to be surprisingly low. Do you use fully activated silica and aluminum?

Mr. Stekoll: Both of them were partially deactivated. We added approximately 6% for alumina, and 5% for the silica gel.

Dr. Rogerson: We have noticed that the heavier aromatics when more fully activated don't move.

Mr. Stekoll: True. We are in the process of redoing our whole extraction procedure for recovery. We have also been having problems with the methylester formation. We eliminated the methanol in the siphonification, and then had problems with emulsions. So we had to extract with benzene instead of hexane, using a centrifuge.

THE BENTHIC COMMUNITY OF A NATURAL OIL SEEP IN THE SANTA BARBARA CHANNEL

Robert B. Spies, Paul H. Davis, and Thomas M. Distler*

Little information is available on the effects of petroleum hydrocarbons on soft bottom benthic communities. Post-spill surveys, such as after the 1969 Santa Barbara Oil Spill (Straughan, 1971) are generally made against a background of insufficient data and, therefore, are only able to detect the most gross effects. A notable exception has been the follow-up work on the West Falmouth Oil Spill (Michael et al., 1975). Often, other complicating factors, such as the extensive use of detergents (Smith, 1970), have made it difficult to separate the effects of petroleum hydrocarbons. Since spills occur unpredictably and are ephemeral in behavior, the systematic study of their effects is extremely difficult. There is usually insufficient background data, and the logistical problems are difficult. Sources of chronic hydrocarbon pollution should provide a more stable setting for studying such effects. In addition, if the best estimates of input rates of petroleum hydrocarbons to the marine environment (National Academy of Science, 1975, p. 6) are examined, it can be seen that chronic sources exceed accidental sources. Therefore, we believe that a thorough study of chronic hydrocarbon pollution of soft bottom communities is needed.

The profuse offshore oil seeps of the Santa Barbara Channel provide an excellent natural laboratory for such a study. Previous work has established the location, nature, and estimates of flow rates of these seeps (Rosen et al., 1959; Wilkinson, 1971; Vernon and Slater, 1963; Mikolaj et al., 1972; Allen and Schlueter, 1969; Allen et al., 1970).

Detailed information provided by Allen et al. (1970) on a small group of profuse seeps near Goleta, California, led us to investigate these as a possible area for our study. After some exploratory dives, we found that one relatively isolated seep, 800 to 900 m off Isla Vista, is located in an area of a homogeneous-appearing sand bottom. The sediments are extensively contaminated with crude oil and, at 15 to 16 m depths, it is shallow enough to employ diving for intensive sampling and observations.

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The only ecological work in the open literature concerning the seep is a report of the absence of ovicell hyperplasia in bryzoans from the vicinity of Coal Oil Point (Straughan and Lawrence, 1975). We report here the preliminary results of a study of benthic community composition in this oil seep and a comparable nonseep area nearby. These are the first results of a study of temporal variation in the Nothria-Tellina community, which is a large-scale feature of the northern Santa Barbara Shelf (Jones, 1969). It is intended that data from the comparison station serve as a measure of natural temporal variation in this community.

METHODS

To find a suitable study site, we made a series of dives in the group of seeps running southwesterly from Coal Oil Point, Goleta, California. The sand overburden in this area was generally thin and variable in water depths to 21 m. Shale outcroppings were common, and it was apparent that extensive sampling would be required to adequately characterize the benthic community. We finally settled on the nearby Isla Vista Seep (C_d in Mikolaj, 1972) because of the larger sand overburden in shallow water and, consequently, more homogeneous bottom. A nonseep comparison station was selected on a similar bottom type approximately 1,408 m (7/8 mile) to the east (Exhibit 1). We are well aware that this second station is not an absolute control. We have seen small amounts of weathered tar in the sediments there, and occasionally a surface slick has coated our buoy. However, we have never seen fresh oil in the cores from the nonseep station, and chemical analyses bear out the large differences in hydrocarbons at the two areas. We did not want to establish the nonseep station too much farther away because of the possibility of other physical and chemical factors influencing community composition. As it is, both stations are 15 m deep, have identical fine sand sediments, and are equidistant from a prominent headland. Our observations are that currents, surge, suspended particulates, and light are also always similar in the two areas.

Each station is marked with a buoy for accurate resampling. We have had trouble losing buoys in storms. Consequently, the original seep station of December 1975 was reestablished in February and again in April 1976, using identical compass fixes on local landmarks. We have been successful in relocating the nonseep station with underwater search techniques and, consequently, it has been continuously sampled. During later reconnaissance, we have relocated the second seep station relative to the present station. It is approximately 53 m west and at the same depth.

We now have a sturdy, all-stainless steel buoy system consisting of a fence post anchor, 3/16 in. cable, and a large foam-filled buoy. The fence post anchor has a 4 in. disc and is screwed at least 65 cm into the bottom. Spatial continuity in seep station samples begins April 1976.

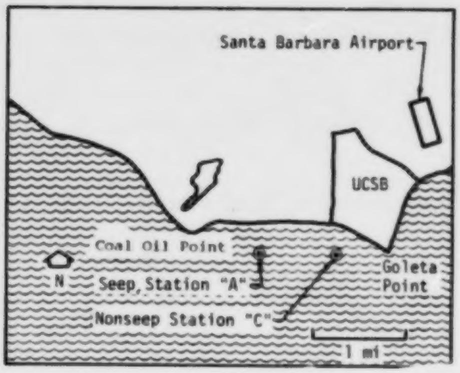

EXHIBIT 1: MAP OF STUDY AREA

Samples are taken within a 50 m^2 area at each station. The area has been subdivided into two hundred 0.25 m^2 quadrates. Every 2 months, 10 quadrates are sampled at each station. The quadrates are selected using a random number table. This system allows us to record the location of every core taken during the study to eventually determine if there are any small-scale spatial patterns in the local community. Our present plans are to sample each station every 2 months for at least 18 months and then to continue sampling on a less rigorous schedule for a number of years.

The samples are vertical cores 0.018 m^2 in area and 20 cm deep. The coring device is a 3 lb coffee can, open at the bottom and punched with a hole in the top to accept a No. 9 rubber stopper. The can is pushed all the way into the sediment, the stopper inserted, and then the whole can is extracted. The open end is capped with a plastic lid. The samples are sea water sieved on a 0.5 mm mesh screen within 4 hours of collection. The sieve contents are then preserved in 10% neutralized formalin and sea water. In the laboratory, samples are washed and elutriated with fresh water, which concentrates most worms and crustaceans in a small volume separate from the sediment. The eluted and sediment fractions are then sorted in large trays under low-power stereomicroscopes. Polychaetes, crustaceans, molluscs, and miscellaneous groups are separated and preserved in a 70% ethyl alcohol-5% glycerine solution.

Biomass determinations were initiated with the February 1976 samples. Determinations are made on each core for each group of rough-sorted organisms. An attempt is made to separate particularly large organisms and weigh them separately. Organisms are removed from the alcohol preservative, blotted dry, air dried for 10 min, and weighed.

All organisms, with a few exceptions, are identified by species and counted for each core.

Community diversity H' is estimated according to Shannon-Weaver (1963) using the approximation:

$$H' = -\sum_{i}^{s} \frac{n_i}{N} \log_2 \frac{n_i}{N}$$

in conjunction with Pielou's measure of evenness J (1966a):

$$j = \frac{H'}{H \max} = \frac{H'}{\log_2 s},$$

where

N = total number of individuals (all species)

n_i = number of individuals of each species

s = number of species.

Smaller, 5 cm diameter cores were used to obtain samples for sediment size-class distribution and hydrocarbon analysis. These are found in about the top 22 to 25 cm of sediment.

For hydrocarbon analysis of sediments, each core is thoroughly mixed and subsampled. The subsample is acid-treated, water-washed, and freeze-dried followed by extraction with toluene-methanol for 300 cycles in a soxhlet apparatus (Rohrback and Reed, 1975). Total extractable material is determined gravimetrically on a microbalance.

Sediment-size-class analyses were performed using sieving and modified Emory settling-tube methods following the method of Buchanan and Kain (1971).

RESULTS

We describe the results of our analysis below.

Hydrocarbon Measurements

The results from April and June 1976 are given in Exhibit 2. These were collected from the current location of the seep station and are not taken from the same exact location as the faunal data reported here; however, they are representative of the differences in hydrocarbon composition of sediments in the two areas. It can be seen that there is a threefold to fivefold difference between the two areas. Excluding one sample with a large tar lamp, the mean values for the two sampling periods from the seep station are very close, and the deviations are only about 20%. The preliminary data also suggest that the sediment hydrocarbons at the nonseep station are more variable with time, perhaps as a result of irregular local fallout of weathered tar.

EXHIBIT 2: SEDIMENT EXTRACTABLES (% of dry sediment)

Seep		Nonseep
April 1976		
0.70 ± 0.03	$\bar{x}$ = 0.67 ± 0.15	0.15 ± 0.01
0.81 ± 0.03		0.15 ± 0.01
0.51 ± 0.03		0.14 ± 0.01
1.83 - 2.77*		0.10 ± 0.01
		$\bar{x}$ = 0.14 ± 0.02
June 1976		
0.79 ± 0.02		0.28 ± 0.02
0.49 ± 0.02		0.17 ± 0.02
0.81 ± 0.02		0.18 ± 0.02
0.56 ± 0.02		0.22 ± 0.02
$\bar{x}$ = 0.66 ± 0.16		$\bar{x}$ = 0.21 ± 0.05

*Tar lump in sample.

Sediment-Size Analysis

The weight loss after drying 24 hr from two small sediment-core samples from each station varied by only 2%. Therefore, the water content is assumed to be essentially the same at each station (25%).

The results of the sediment-grain-size analysis are given in Exhibit 3 for these four samples. As can be seen, the sediments are very similar. There is about a 5% to 7% difference in the amount of very fine sand (125 to 62μ), probably because of the slight skewing by small bits of weathered tar in the upper size ranges of the seep sediments. This analysis confirms our earlier subjective judgments of the similarity of the two stations.

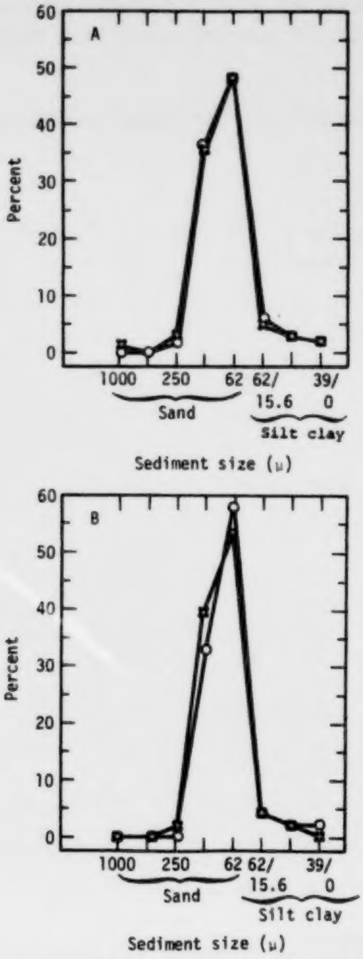

EXHIBIT 3: SEDIMENT SIZE DISTRIBUTION AT SEEP (A) AND NONSEEP (B) STATIONS

Biological Measurements

The December and February samples have been completely processed. The number of species, individuals, and diversity for both stations and sampling periods are given in Exhibit 4.

EXHIBIT 4: COMMUNITY PARAMETERS SEEP (NONSEEP)

	Dec 1975	Feb 1976	Total
No. of species	153 (126)	106 (103)	192
No. of individuals	2900 (1302)	1823 (1264)	4723 (2565)
Total individuals	4202	3087	
Diversity (H')	3.70 (3.72)	3.59 (3.52)	
Evenness (J)	0.74 (0.77)	0.77 (0.76)	

A number of trends are immediately evident:

1. The number of individuals is double at the seep station.
2. The number of species is about 15% higher at the seep station for both sampling periods.
3. There is a sharp reduction in both species and individuals at both stations in February 1976. In addition, the mean biomass at the seep station, 1.53 ± 0.85 g, is higher than at the nonseep station, 1.02 ± 0.47 g (February 1976), but these differences are not significant ($p = 0.05$).

The relationship between the number of individuals and the number of species combined for both sampling periods and for each station is shown in Exhibit 5. It can be seen that 90% of all species so far recorded are collected in 9 cores at the seep station and 10 cores at the nonseep station. Therefore, we believe that our samples adequately represent the community.

A further measure of the differences between the two areas is the number of common species that show increases in the seep-station samples. A common species is defined as one occurring in 50% or more of the cores. For interstation comparisons in this paper, only common species that occur at both stations are used. Thus, out of a total of 202 species, there are 103 species that occur at both stations for data combined from both sampling periods. Of these 103, there are 35 common species at the nonseep station and 64 at the seep station. For December, there are 26 co-occurring common species, and for February there are 22 co-occurring common species. Fifteen of these are the same species. Of the 26 co-occurring common species in December, 21 (81%) have greater densities at

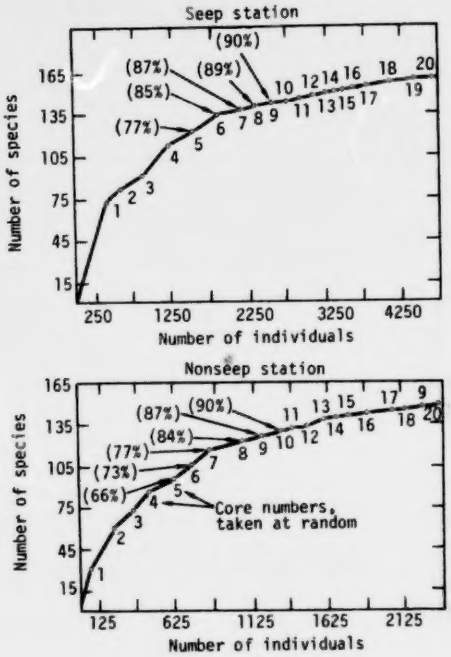

EXHIBIT 5: DISTRIBUTION OF INDIVIDUALS AND SPECIES IN COLLECTIONS FROM BOTH STATIONS [( ) = Cumulative % of total species]

the seep station, and one species has the same. In February, there were 22 such species with 17 (77%) having greater densities at the seep station and one species having the same.

To compare the two stations further, a Spearman rank correlation analysis was performed on the 26 co-occurring common species from December. The results indicated a significant ($p = 0.01$) positive correlation ($r_s = 0.64$). A similar result was observed for the February data, thus supporting the hypothesis that the community structures of the two areas are basically the same.

A list of the 10 most common species from each station in the December samples is given in Exhibit 6. Included are the mean densities, standard errors, and an estimate of the number of samples required to achieve a 20% degree of precision (Elliott, 1971). This last measure is an indication of which species will be amenable to more detailed population studies under the current sampling scheme.

Of the 10 most common species listed for the seep station in Exhibit 6, the following species showed a 300% or more increase over the control station: Mediomastus californiensis, Tellina modesta, Oligochaetes, and Praxillella affinis pacifica. These are all deposit feeders.

EXHIBIT 6: TEN MOST ABUNDANT SPECIES (TAXA) FROM DECEMBER 1975

Species	Density/Core	$n = \frac{s^2}{D^2 \bar{x}^2}$, (D = 0.20)*
A. Collected at seep station	29.8 ± 9.22	2.4
1. *Mediomastus californiensis*	29.2 ± 13.5	5.3
2. *Tellina modesta*	30.7 ± 20.2	10.8
3. Oligochaetes	22.3 ± 14.3	10.2
4. Nematodes	18.7 ± 10.9	8.5
5. *Chaetozone neosetosa*	10.0 ± 7.9	15.7
6. *Prionospio pygmaeus*	9.8 ± 6.6	11.2
7. *Praxillella affinis pacifica*	9.4 ± 4.1	4.8
8. *Euphilomedes* sp.	8.1 ± 5.9	13.3
9. *Nepthys caecoides*	6.6 ± 3.9	8.7
10. *Lumbrineris tetraura*		
B. Collected at nonseep station		
1. *Prionospio pygmaeus*	16.7 ± 6.1	3.3
2. Nematodes	14.3 ± 9.3	10.4
3. *Chaetozone neosetosa*	8.6 ± 4.0	5.3
4. *Mediomastus acutus*	7.6 ± 3.5	5.2
5. *Mediomastus californiensis*	7.4 ± 3.3	5.0
6. *Tellina modesta*	6.1 ± 5.0	16.9
7. *Euphilomedes* sp.	4.9 ± 4.6	22.3
8. *Nepthys caecoides*	4.2 ± 2.1	6.2
9. *Paraphoxus daboius*	3.7 ± 1.8	5.7
10. *Exogone uniformis*	2.8 ± 2.9	26.8

*Elliott, 1971.

Although the polychaete worm *Diopatra ornata* is not among the most common species, its tubes are a prominent aspect of the bottom relief. In the February 1976 samples from the seep station, it constituted 34% of the biomass, which suggests that it is an energetically important species. Its presence in a core sample also has a demonstrable enriching effect on the number of individuals and species (see Exhibit 7). Here, there are significant ($p < 0.01$) differences in the numbers of individuals and in the numbers of species ($p < 0.001$) (t-test).

EXHIBIT 7: THE EFFECT OF *DIOPATRA* ON NUMBERS OF INDIVIDUALS AND SPECIES: SEEP STATION

	No. of Individuals, $\bar{x}$	No. of Species, $\bar{x}$
Samples with *Diopatra* n = 4	355 ± 42	70 ± 5
Samples without *Diopatra* n = 6	246 ± 50*	49 ± 6†

*Significantly different at $p = 0.01$ (t-test).

†Significantly different at $p = 0.001$ (t-test).

Bacterial Mats

Large white mats of the bacterium *Beggiatoa* sp. are commonly seen associated with particularly active seepage (see Exhibit 8). The mats are usually only a few millimeters or less in thickness. They are often a meter or more in diameter and may even extend in an irregular pattern over larger areas of seep during calm weather.

Beggiatoa is known as a H_2S oxidizer, but it requires aerobic conditions. These conditions appear to exist near areas of most active seepage. The black, anaerobic layer in the sediment rises to the sediment-water interface in these areas. The strong smell of H_2S exists in all our core samples; there seems to be little doubt this compound is abundant.

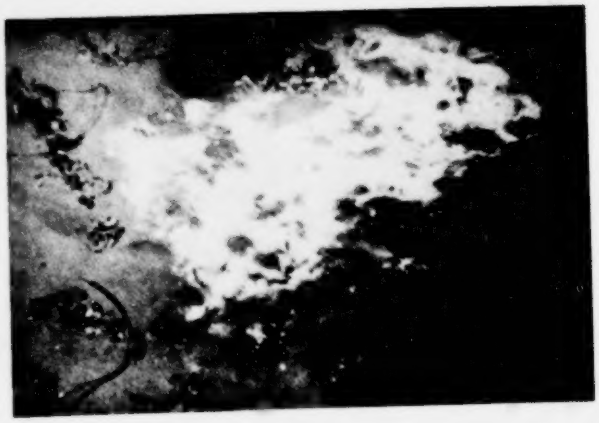

EXHIBIT 8: *BEGGIATOA* MAT GROWING ON AREA OF ACTIVE SEEPAGE [Fish in foreground (*Citharicthys* sp.) is about 12 cm long]

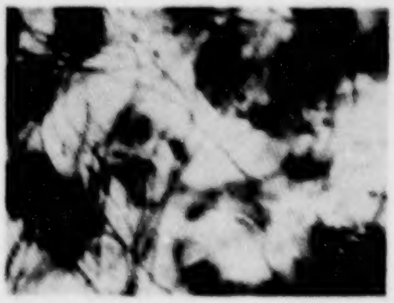

EXHIBIT 9: PHOTOMICROGRAPH OF BEGGIATOA SAMPLE FROM MAT (150 x)

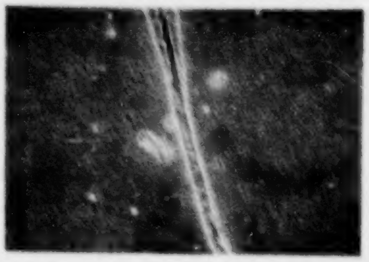

EXHIBIT 10: PHOTOMICROGRAPH OF BEGGIATOA FILAMENT [Note inclusions (400 x)]

sediment-water interface in these areas. The strong smell of H_2S exists in all our core samples; there seems to be little doubt this compound is abundant.

The filamentous cells of Beggiatoa (see Exhibits 9 and 10) are very motile and are probably able to position themselves at the Eh discontinuity boundary.

We have seen small nematodes within the mat, but when we attempted to core a mat the cores had such large amounts of oil that we were unable to sieve them to determine if other organisms were present.

Other Biological Observations

In the course of many dives in the two areas, we have the strong impression that the larger epibenthic organisms that are not usually captured in our samples are generally more abundant in the seep area. These include the asteroids Pisaster brevispinous, Patiria miniata, Astropectin armatus, and A. californicus; the neogastropod mollusc Kelletis kelletii; and the fish Citharicthys sp and Hyperprosopon argenteum. These last two species seemed to be attracted to our activities, so it is difficult to judge their true abundances. Both Hyperprosopon and Astropectin were quickly drawn to the depressions left from our coring where they appeared to be actively feeding on exposed organisms.

DISCUSSION AND CONCLUSIONS

The consistent differences in most measured biological parameters in the seep area over the nonseep area seem conclusive. The seep area does support an enriched community. Most major physical parameters (i.e., depth, sediment type, surge, distance from shore, suspended particulates, and light penetration) appear to be about the same in the two areas. It is possible that some factor that is not observed or measured, such as nutrient concentrations, could be influencing these areas unevenly; however, it seems unlikely. Therefore, we hypothesize that the constant presence of fresh crude oil in the sediments is responsible for the enriching effect.

If this is accepted, then there are a number of further questions:

- Why are the toxic effects of the oil not more apparent?
- How can crude oil enrich a benthic community?

To speculate about these questions briefly, it seems to us that either the toxic compounds are not in solution at toxic levels in the interstitial and overlying water or else the benthic organisms in the seep area have developed the biochemical pathways to detoxify these compounds. The latter possibility seems likely with the increasing body of evidence that points to the presence of enzyme systems capable of metabolizing aromatic compounds (Payne and Penrose, 1975; Payne, 1976; Corner et al., 1976; Lee, 1975).

As to the enriching effect, the abundance of bacteria in association with the active seepage suggests that petroleum hydrocarbons are being utilized as an extra carbon source. Although Beggiatoa has been characterized as a chemautotroph (Zajic, 1969), it has been shown to utilize acetate (Burton et al., 1966). Therefore, the possibility of these large mats utilizing some portion of the oil remains open. The origin of the H_2S may be recent or perhaps in the formation water that could be accompanying the oil. The increased abundance of deposit feeders might then be attributable to increased microbial abundance.

Clearly, this is all speculation and further observations. Measurements and experiments are needed to clarify the effects and fate of petroleum hydrocarbons in this benthic community.

Work in Progress

A number of separate but related efforts are currently in progress.

- The benthic sampling program will be continued until 18 months of data are available for analysis. This should indicate the type of continuing sampling effort needed to define a baseline of natural temporal variation. It will also allow study of comparative population dynamics at the two stations.
- Approximately 30 low molecular weight hydrocarbons comprise the majority of the crude seep oil. We will be characterizing these and developing methods for determining these in water, sediments, and organisms.
- The reproductive cycles of two populations of echinoderms thought to be impaired in the vicinity of the seep will be monitored.
- Attempts are being made to demonstrate acclimation phenomena by comparative toxicity of water of soluble fractions of crude oil to developmental stages of the starfish *Patiria miniata*. Reproductively active adults from the seep and a relatively pristine control will be used.

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DISCUSSION

Dr. Petrocelli: In the area of chronic oil seeps, have you observed any abnormal growths in any of the indigenous species similar to some of the tumors that have been found on flat fish in that area?

Dr. Spies: We have not really studies these abnormal growths, but while we were conducting the experiments fishermen mentioned organisms with strange growths.

Dr. Petrocelli: Burns and Sherwood and others have reported various types of anomalous growths and fin erosion in the fishes that contact the bottom at Quellelle Point and further south.

Dr. Spies: Of course, there is the possibility that the solubility of hydrocarbon is low here. Several organisms feed on the benthic community in that area, such as cythruictes and the phenerodon, a common surf perch. It may be that they are acclimated to the presence of this stuff. They could have enzyme systems in their liver and so forth.

From the floor: Do you plan to use a Tellis analysis?

Dr. Spies: I think that the Denver ground-type of approach would be more appropriate for a multistation type of study. We have only two stations.

From the floor: When you add the species, it should break out in a whole bunch of different ways. The co-occurrence of those would be the interesting part.

Dr. Spies: Although we intend to get into this problem, we have not examined appropriate methodologies for looking at the bulk of this data. We are particularly interested in reproductive cycles of organisms common to both areas, recruitment, and this kind of thing.

Dr. Davis: Can you speculate to what degree sediment oil is reintroduced by the water column, or does it come from geological seeping? Are there any important oils in the sediment that are coming from the water?

Dr. Spies: So far, we have not made any water column measurements. Several investigators have been measuring high molecular weight compounds in the water; at a nearby station they have a level of N-alkanes in ppb, an order of magnitude higher than most of southern California, which has parts per trillions. The question of the solubility of this oil and water is open right now.

ACUTE AND CHRONIC STUDIES WITH WATERFOWL EXPOSED TO PETROLEUM HYDROCARBONS

Michael P. Dieter*

Our requirements for energy and economic independence will rest largely on petroleum hydrocarbons for several decades. This recognition has resulted in concerted efforts to find, obtain, process, and deliver oil at an ever-increasing rate. Coupled with this phenomena is our nation's awareness and dedication to the maintenance of ecological balance. Many government agencies have been assigned the task of ensuring our wildlife heritage during the removal of nonliving natural resources. We represent a small part of this effort: an examination of the ecological and physiological/toxicological effects of oil on birds. Our studies, which encompass numerous aspects of avian biology, are proceeding on a broad front as fast as possible in an attempt to answer one basic question: do sublethal concentrations of petroleum hydrocarbons pose a significant threat to bird populations?

The effects of petroleum hydrocarbons on aquatic birds have been the subject of two recent symposia (Dieter, 1976; Szaro, 1977). These reviews pointed out that the base of scientific information was scant and needed expansion. Nevertheless, the available evidence was alarming and indicated the potential damage that petroleum hydrocarbons might inflict on susceptible avian populations.

The research program at Patuxent has been evaluating the biological effects of petroleum hydrocarbons on all stages of the waterfowl life cycle. These studies include: (1) the hatchability of eggs exposed to oils; (2) the development of ducklings fed oil from the day of hatch; and (3) reproduction in adult waterfowl fed oil. The third study is complemented by another where adult waterfowl were fed a chemically defined mixture representative of the concentration and type of aromatic compounds present in oil. In addition, experiments have been initiated to evaluate biological responses to petroleum hydrocarbons incorporated via the food chain. Analytical development is proceeding in concert with all of these biological studies.

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We have repeatedly demonstrated that μℓ amounts of crude or refined petroleum hydrocarbons result in high mortality of developing embryos. Exhibit 1 shows that 5 μℓ of South Louisiana crude oil, Kuwait crude oil, or No. 2 fuel oil, applied to fertile eggs at 8 days of incubation, caused 76-98% mortality. We have also concluded that mortality is a result of toxicity and not oxygen deprivation, and that an alkane mixture tested was nontoxic, implicating the aromatic fraction or other constituents of crude oil as the toxic components.

EXHIBIT 1: EMBRYOTOXICITY FROM EGG OILING* WITH DIFFERENT PETROLEUM PRODUCTS (N=50)

	Percent Mortality after Treatment with:		
Treatment	South La. crude oil	Kuwait crude oil	No. 2 fuel oil
None	8	8	12
Propylene glycol (50 μℓ)	6	-	-
Alkane mixture (50 μℓ)[†]	4	-	-
Oil (1 μℓ)	38**	28**	36**
Oil (5 μℓ)	98**	76**	82**
Oil (10 μℓ)	98**	84**	90**
Oil (20 μℓ)	100**	94**	100**

Note: Data derived from the studies of Szaro, Albers, and Coon (1977).

* Fertile mallard eggs treated externally at day 8 of development.

† See Exhibit 6 for components of alkane mixture.

** Significantly different from controls, $P < 0.01$.

The embryotoxicity of oils was not confined to the mallard. Eider eggs collected from the Maine seacoast were externally oiled with No. 2 fuel oil resulting in 31% mortality (see Exhibit 2). Eider embryos appeared to be 1/20 as sensitive as mallard embryos to external egg oiling. Some of the species difference can be ascribed to different dose-weight relationships, as the eider embryos are twice the size of mallard embryos. Age-dependent sensitivity to petroleum hydrocarbons is another factor that was responsible (see Exhibit 3). Embryos that were at earlier stages of development were much more sensitive to egg oiling than later stages. This was supported by another series of experiments shown in Exhibit 4.

EXHIBIT 2: EMBRYOTOXICITY IN OILED COMMON EIDER EGGS* (N=48)

Treatment	Percent Mortality
None	4
Propylene glycol (50 μℓ)	4
Oil (5 μℓ)	8
Oil (20 μℓ)	31†

Note: Data derived from the studies of Szaro and Albers (1977).

* Fertile eider eggs of various developmental stages treated externally with No. 2 fuel oil.

† Significantly different from controls, $P < 0.01$.

EXHIBIT 3: AGE-DEPENDENT EMBRYOTOXICITY* TO OIL IN COMMON EIDER EGGS

No. 2 Fuel Oil (20 μℓ)	N	Age in Days (Mean ± S.E.)
Dead	15	4.3 ± 1.4
Survivors	33	16.1 ± 0.7

Note: Data derived from the studies of Szaro and Albers (1977).

* Significantly different ages, $P < 0.05$.

EXHIBIT 4: EMBRYOTOXICITY FROM EGG OILING* AT DIFFERENT DEVELOPMENTAL STAGES (N=50)

Age of Treated Embryo (days)	Percent Mortality after Treatment with:	
	South Louisiana Crude Oil	No. 2 Fuel Oil
Controls†	0	20
2	100**	87**
6	97**	67**
10	92**	32**
14	22**	17
18	12**	20
22	5	7

Note: Data derived from Albers (1977).

* Fertile mallard eggs treated externally with 5 μℓ of oil.

† Mortality of untreated control eggs checked by candling at each interval.

** Significantly different from controls, $P < 0.05$.

Mortality from petroleum hydrocarbon application was clearly age dependent; 5 μℓ applied on or before 10 days of development resulted in 92% or greater mortality from South Louisiana crude oil and 32-87% mortality from No. 2 fuel oil (see Exhibit 4). Treatment of eggs on or beyond day 14 resulted in much less mortality. This line of investigation will be continued with eggs from different species and with other crude oil products, and will be extended by conducting field studies with eggs oiled in marked nests and outdoor pen studies with oiled waterponds and naturally incubating hens.

We also found that hens from paired mallards fed 2.5% South Louisiana crude oil laid fewer eggs than pairs fed 0.25%, a mixture of 1% paraffins, or untreated control pairs. Egg production for 30 days dropped from an average of 25 eggs/hen in untreated to 11 eggs/hen in those fed 2.5% South Louisiana crude oil. Our contract investigator has reported similar results with mallards fed 1%, 3%, or 5% South Louisiana crude oil (Holmes, personal communication). He reported that hens fed 3% oil ceased laying, and that those fed 1% oil laid one-third as many eggs as controls. It is evident that, during the reproductive season, petroleum hydrocarbons could pose a threat to waterfowl populations by interfering with the production and viability of eggs.

EXHIBIT 5: MORPHOLOGICAL AND BIOCHEMICAL RESPONSES IN MALLARD DUCKLINGS FED SOUTH LOUISIANA CRUDE OIL FROM HATCH TO 8 WEEKS OF AGE (MEANS ± S.E., N=10)

Morphology

Concentration of Oil Fed	Body Weight	Liver Weight		Spleen Weight	
(ppm)	g	g	% BW	g	% BW
0	1119	34.5 ± 1.5	3.2	0.81 ± 0.06	0.074
250	1127	33.0 ± 1.9	3.0	0.84 ± 0.12	0.067
2500	1109	31.5 ± 1.7	2.9	0.64 ± 0.04	0.058*
25000	1069	51.5 ± 1.9	4.8*	0.39 ± 0.06	0.037*
50000	913	69.7 ± 4.7	7.4*	0.30 ± 0.03	0.032*

Biochemistry†

Plasma Enzyme Activity

	Alanine aminotransferase (from liver) milliunits	Ornithine Carbamyl transferase (from kidney) units	α-hydroxybutyric acid dehydrogenase (from heart) milliunits
0	11.6 ± 1.0	12.5 ± 0.6	96.4 ± 9.0
250	12.7 ± 2.1	23.1 ± 1.2*	112.3 ± 8.9
2500	23.5 ± 3.3*	19.0 ± 1.8*	84.6 ± 7.8
25000	19.0 ± 1.6*	18.8 ± 1.1*	77.8 ± 7.7
50000	16.3 ± 1.4*	20.7 ± 1.5*	86.3 ± 12.5

Note: Data derived from Szaro (unpublished data, 1977).

* Significantly different from controls, $P < 0.05$.

† Additional plasma enzymes measured that showed no significant change included aspartate aminotransferase, lactate dehydrogenase, and cholinesterase.

Duckling growth was impaired when diets containing 5% South Louisiana crude oil were ingested from hatching until 8 weeks of age (see Exhibit 5). More importantly, in these ducks, as well as those fed 2.5% South Louisiana crude oil, flight feathers failed to develop normally. Liver hypertrophy and splenic atrophy were evidence of the pathological effects of the oil; biochemical lesions that occurred included elevation of plasma alanine aminotransferase and ornithine carbamyl transferase activity. These plasma enzymes appear in the circulation as a result of liver and kidney damage.

The same enzymes are being measured in studies with adult ducks fed South Louisiana crude oil or representative aromatic compounds of this crude oil. These variables have thus far failed to reveal dysfunction in adult ducks fed up to 2.5% South Louisiana crude oil, or in those fed the aromatic mixtures.

More sophisticated measurements of liver function were performed with the drakes fed the aromatic mixture. The ability of the liver to remove toxic substances from the circulation was followed by measuring the change in the clearance rate of injected indocyanine green dye, a compound that is metabolized entirely by the liver. The ingestion of the 4000 ppm aromatic mixture caused a compensatory response to the toxicants reflected by a significant elevation of plasma clearance rates above control levels (see Exhibit 6). The data suggest that adult waterfowl may be able to adapt and tolerate higher concentrations of petroleum hydrocarbons than ducklings.

Thus, our present evidence indicates that all phases of the reproductive sequence (i.e., fertilization, laying, and embryonic and duckling development) are highly sensitive to petroleum hydrocarbons. Hopefully, extensive measures could be undertaken to prevent oil exposure during this critical period.

In other studies, waterfowl were fed various invertebrates that had been exposed to petroleum hydrocarbons to determine the nature and extent of the products accumulated in their tissues. Crayfish have been been selected as food items that are readily accepted by mallard ducks. A radiolabeled aromatic compound is used as a marker to follow the oil through the food chain.

Along with the biological work, a concerted effort has been made to develop analytical capability for petroleum hydrocarbons. This poses a special challenge because of the overwhelming number of chemical compounds present in one sample, and the fact that this array is unique for different oils and even for different batches of the same oil.

The first problem addressed was the demonstration of the accumulation of petroleum hydrocarbons in avian tissues. High-resolution gas chromatography and mass spectrometry were used to identify the petroleum-derived, saturated hydrocarbons in tissues of oil-dosed ducks (Lawler, et al., 1977). The distribution pattern of n-alkanes, the homologous series of petroleum hydrocarbons, and the $\underline{n}$-C_{17}/pristane ratio were used to provide evidence that petroleum hydrocarbons had accumulated in the tissues. Selective uptake and/or metabolism had apparently occurred since tissue levels of individual saturated petroleum hydrocarbons differed from the relative amounts present in South Louisiana crude oil. No accurate quantitation of saturated petroleum hydrocarbons was possible, but relative accumulation by tissues

EXHIBIT 6: LIVER FUNCTION IN MALLARD DRAKES FED AROMATIC MIXTURES*
REPRESENTATIVE OF THOSE IN SOUTH LOUISIANA CRUDE OIL
(MEANS ± S.E., N=12)

		Months		
	Pretreatment	1	3	5
Treatment	Plasma Clearance Rate (ml/min/kg body weight)			
Control	13.7 ± 1.1	13.5 ± 0.8	13.5 ± 0.7	13.1 ± 0.7
Alkane mix, 10,000 ppm	14.0 ± 1.1	14.7 ± 0.7	14.9 ± 0.7	13.1 ± 0.7
Aromatic mix, 400 ppm	13.4 ± 1.0	15.5 ± 0.8	15.2 ± 0.7	14.7 ± 0.5
Aromatic mix, 4000 ppm	14.1 ± 1.0	17.0 ± 1.4[†]	18.5 ± 1.0[†]	18.5 ± 1.2[†]

Note: Data derived from Patton (unpublished data, 1977).

* The aromatic mixture consists of an equimolar ratio of 10 compounds (ethyl benzene, 1,2,3,4-tetrahydronaphthalene, dimethylnaphthalene, 2, 3-trimethylindolenine, acenaphthylene, acenaphthene, phenanthrene, 2-methylbenzothiazole, dibenzothiophene, and 2,6-dimethylquinoline) dissolved in 1% alkanes (equimolar mixture of tridecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4,6,6-pentamethyl heptane, 2,2,4,4,6,8,8-heptamethylnonane, 2,6,10,14-tetramethylpentadecane, and decahydronaphthalene) in the feed.

† Significantly different from controls, $P < 0.05$.

was: skin > uropygial gland > breast muscle > heart muscle > liver > blood. No saturated petroleum hydrocarbons were detectable in the brain and only trace amounts in the blood.

The unsaturated petroleum hydrocarbons (aromatic compounds) are now being measured in the same tissue extracts. The tissue accumulation of aromatic hydrocarbons was similar to that of the alkanes, except that detectable quantities of aromatics were present in the brain.

Our analytical efforts favor the aromatic fraction because the biological evidence indicates that the aromatic compounds in petroleum hydrocarbons are toxic. We are streamlining present analytical methods and are selecting marker compounds in petroleum hydrocarbons to serve as references for quantitation. Pending completion of these tasks, tissues from mallard ducklings fed South Louisiana crude oil are scheduled for petroleum hydrocarbon analysis. Other material that will soon become available for analysis includes tissues from adult mallards fed either South Louisiana crude oil or an aromatic mixture representative of this oil.

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DISCUSSION

Dr. Anderson: Have you tried the oiling of the eggs with weathered crude, because of the aromatic decrease?

Dr. Dieter: In our current series of experiments, we are using Alaskan crude oil and weathered and unweathered oils. We are also going to use some water-soluble fractions to investigate whether we can find a decrease in embryo toxicity. 50,000 parts commutes to a very high physiological or pharmacological dose.

Dr. McErlean: Do you have any feeling of the effects of an oil spill?

Dr. Dieter: I would speculate that we would be looking at responses from ducks at the level of 250 or perhaps 2,500 ppm; 50,000 ppm is definitely a pharmacological level. We wished to bracket the levels where we might see an effect, and then work down to make sure that we did not miss anything. In these chronically fed birds, 250 ppm is approximately 2.5 mg a day ingested per duck; in the case of ducklings it is over an 8-week period.

Dr. Engel: What was the label compound used?

Dr. Dieter: We began with C-14 naphthalene and will try other labels. So far, we have not performed any metabolite studies. We have calculated the DPMs and the rough ppm naphthalene that can be surmised from the disintegrations in the tissue. This is over 1 ppm in the gall bladder of the ducks fed the radio-labeled crawfish, a two-fold and then a three-fold increase in the radio-labeled compound in the fat, a steady increase in the kidney, and a slower increase in the liver. From this food chain study, the gall bladder is the highest accumulator so far, although that is probably all metabolite; the fat is the next highest, and then the kidney and the liver.

Dr. Kunhalt: When did you check whether the eggs were dead or did you count the hatchlings or surviving ducks?

Dr. Dieter: The majority mortality resulting from the external exposure of the mℓ amount of oil occurs within 6 days after exposure. The eggs are checked every other day for mortality by candling. If they appear to be dead, then they are opened to verify this conclusion.

Dr. Kunhalt: I am not familiar with the development stages in ducks. You mentioned, first, the application of 8 days and showed the mortalities. What stage is that?

Dr. Dieter: It is a full embryo. For the blastula stage it is below the first day.

ACCUMULATION AND BIOLOGICAL EFFECTS OF COPPER AND CADMIUM ON MARINE SHELLFISH

David W. Engel* and Bruce A. Fowler†

In recent years, the interface between research related to human health and ecological research has become much less distinct. The interplay between these two areas of research has become more relevant because of our expanding technology and greater demand for electric power. As a result of our increased demand for energy, there has been an increase in trace metals entering the waters of estuaries and oceans. Some of these metals come directly from energy production (i.e., the burning of fossil fuels), from the cooling process (i.e., corrosion products from condenser tubes), or from industrial and municipal waste. These metals can accumulate in marine fish and shellfish, which may alter their capacity to withstand natural environmental stresses. In addition, these organisms may become food for human consumption, and the accumulated metals will enter the human diet.

The research that we are conducting concerns the effect of copper and cadmium on the metabolism of shellfish. Copper may enter the environment from power plants and industrial sources, while cadmium enters primarily from industrial waste and sewage effluents.

In most of the pharmacological investigations concerned with the toxic effects of metals on mammals, the metal is added to the diet as a mineral salt. However, most dietary metals are present as organocomplexes, and these different chemical species may drastically affect their toxicological and pharmacological properties. In our laboratory, we have demonstrated that the various chemical species of copper can control the toxic effects of the metal on marine fish eggs and larvae and algae (Engel et al., 1976; Sunda and Guillard, 1976; Sunda, 1975). To approximate acute pollution situations, shellfish are being labeled with metals (copper and cadmium) over extended periods of time, and the labeled meat will be used in the production of a "toxic" rat diet that represents more nearly a natural situation.

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The two objectives of our research are:

1. Label shellfish with biologically incorporated copper (Cu) and cadmium (Cd) that will be used in the preparation of rat diets for pharmacokinetic and toxicity investigations

2. Determine physiological and fine structural changes in marine shellfish caused by copper and cadmium.

Experimental Design

The flowing water system, which was constructed for metal exposures, is similar to the system being used for exposure of organisms to pesticides at the Environmental Protection Agency, Gulf Breeze Pesticide Laboratory (Bahner, et al., 1975). The primary difference between the two systems is that we use ambient sea water.

Sea water is pumped through a coarse filter into the 640 ℓ recirculating temperature control tank (Living Stream Unit),* where it is filtered again through activated charcoal, and heated or cooled to maintain a constant temperature (see Exhibit 1). Water is then pumped from the temperature control tank up to a 95 ℓ polyethylene reservoir, which functions as a constant head tank.

The water-toxicant distribution system, experimental tanks, flow meters, pipes, tubing, and syringe pumps are supported by a heavy-duty wood frame with two 3 in. deep trays. The insides of the trays are fiberglass lined, and each tray is connected to a drain. Two and five 38 ℓ experimental tanks can be accommodated on the upper and lower levels, respectively. Gilmont flowmeters were used to monitor the flow of diluent water from the manifold to the tanks, and the flow rates may be set between 5 mℓ and 350 mℓ per minute.

Sage syringe pumps are used to inject the toxicant at rates that can be varied from 4×10^{-4} mℓ to 140 mℓ/min. Each pump holds from one to three glass or plastic syringes, depending on size. Thus, either one 100 mℓ syringe, two 50 mℓ syringes, or any three syringes up to 30 mℓ can be used. Toxicant and diluent water flow separately through appropriate plastic tubings to mix in a glass funnel before flowing into the experimental tanks. Test water flows out of the experimental tanks through glass T-joints cemented in the side ends of the tanks.

Using the flowing water exposure system described above, the oysters were labeled separately with copper or cadmium. The oysters

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* The use of trade names does not constitute an endorsement of these products by National Oceanographic and Atmospheric Administration (NOAA) or the National Marine Fisheries Service (NMFS).

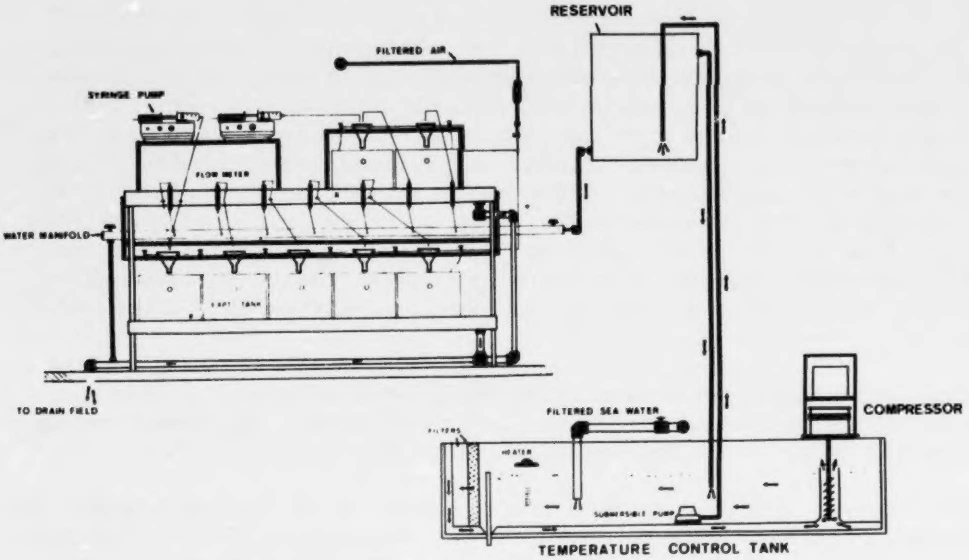

EXHIBIT 1: A FLOWING WATER BIOASSAY SYSTEM, WHICH IS BEING USED TO DETERMINE THE TOXIC EFFECTS OF METALS ON MARINE FISH AND SHELLFISH (Arrows indicate the pattern of water flow in the system.)

were exposed for 14 days at 0.1 ppm concentration of either copper or cadmium. During the exposure period, the oysters were fed twice a week with mixed algal suspensions, which were spiked with 0.1 ppm of copper or cadmium. At the end of the exposure period, the meats were removed, freeze dried, and used in preparation of rat diets for mammalian pharmacokinetic studies. The levels of cadmium in the oysters ranged from 500 ppm to 1000 ppm dry weight, and the mean concentration of copper was 360 ppm.

EFFECTS OF COPPER ON SHELLFISH

To determine the physiological effects of copper on shellfish, we measured shellfish gill tissue respiration rates. Four species of lamellibranch mollusks were used in the determinations: the American oyster, Crassostrea virginica; the hard clam, Mercenaria mercenaria; the bay scallop, Argopecten irradians; and the ribbed mussel, Modiolus demissus. All animals were collected in the vicinity of our laboratory at Beaufort, North Carolina, and maintained in the laboratory in flowing sea water.

The copper solutions used in the experiments were all buffered with 5 mM "Tris" in 30 ppt salinity Sargasso Sea water at pH 8.0 ± 0.05.

The total copper concentrations of the solutions were 2×10^{-5}M, 5×10^{-5}M, 1×10^{-4}M, and 5×10^{-4}M with cupric ion concentrations of $10^{-8.7}$M, $10^{-8.3}$M, $10^{-8.01}$M, and $10^{-7.15}$M. The control seawater contained "Tris" and a copper concentration of $<10^{-8}$M (Chester and Stoner, 1974), which would give a cupric ion concentration of $<10^{-10}$M. The cupric ion concentrations were derived from the apparent stability constants for copper in seawater (Sunda, 1975). The organic buffer system concept used with copper will reduce the complexation of copper by natural organics in the exposure media, because of the high ratio of total copper to free cupric ion (10,000 to 1). This method allows the prediction of a specific chemical species of copper as the toxic agent (free cupric ion) and results in reduced variability between and within experiments.

The measurements of tissue respiration were made with a Gilson differential respirometer using standard techniques. All measurements are reported as µℓ of oxygen consumed per mg dry weight per hr.

The excised gill tissue from the four species of mollusks exhibited species-specific responses to copper. The respiration rates of scallop and clam tissue increased significantly with increased cupric ion concentrations. Gill tissue from the mussel, however, had a relatively smaller increase in respiration than the scallop or clam tissue, and the respiration rates of oyster gill tissue were not significantly affected by the presence of copper (see Exhibit 2). The response of the clam gill to copper was roughly linear, and the variation within each concentration was quite low. Scallop tissue, however, had exceedingly variable respiration rates at cupric ion concentrations above $10^{-8.3}$M. The primary source of the variation was the copper-induced breakdown of the tissues, which resulted in variable tissue weights. It is interesting to note that the scallop was also the most sensitive to copper in preliminary toxicity tests.

When the respiration data were normalized to the control respiration rates, a clearer picture of species similarities and differences relative to the effect of copper was demonstrated (see Exhibit 3). These responses fall into two categories: the scallop and clam tissue had increased respiration with increased copper concentration, while the mussel and oyster gill had increased respiration at cupric ion concentrations between $10^{-8.7}$M and 10^{-8}M followed by decreased respiration. These differences may be related to the salinity tolerance of the species, with mussels and oysters more euryhaline than either clams or scallops. The observed increase in respiration may be related to copper-induced changes in membrane permeability to ions (Na^+ and K^+) as has been demonstrated with algae cells (McBrien and Hassall, 1965). There also may be copper-mediated changes in mitochrondrial membranes causing uncoupling of the electron transport systems. This possibility is currently under investigation as part of this research program. The observed effects of cupric ion on molluscan gill tissue are in the same range as toxic effects of free cupric ion on spot egg and larvae and unicellular algae (Engel et al., 1976; Sunda and Guillard, 1976).

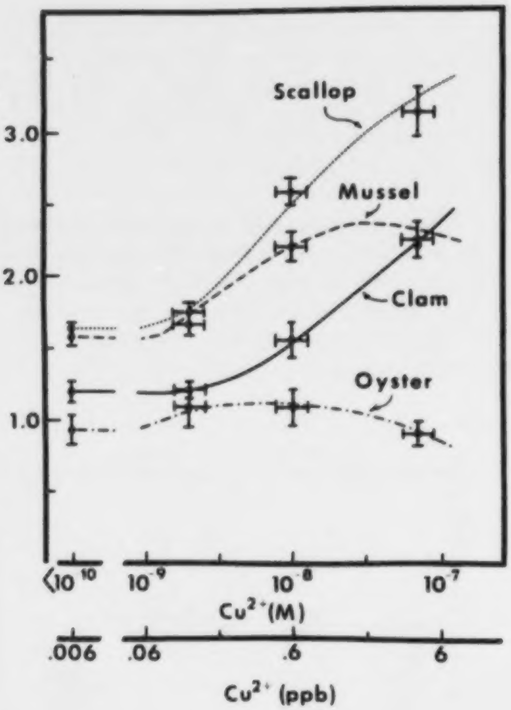

EXHIBIT 2: CHANGES IN MOLLUSCAN GILL TISSUE RESPIRATION RATE AS A FUNCTION OF THE CUPRIC ION CONCENTRATION (Concentration of cupric ion given both in molar units and as ppb. Vertical bars indicate one standard error of the mean respiration rate, and the horizontal bars indicate the range in cupric ion concentration. There are 10 replicates for each mean value.)

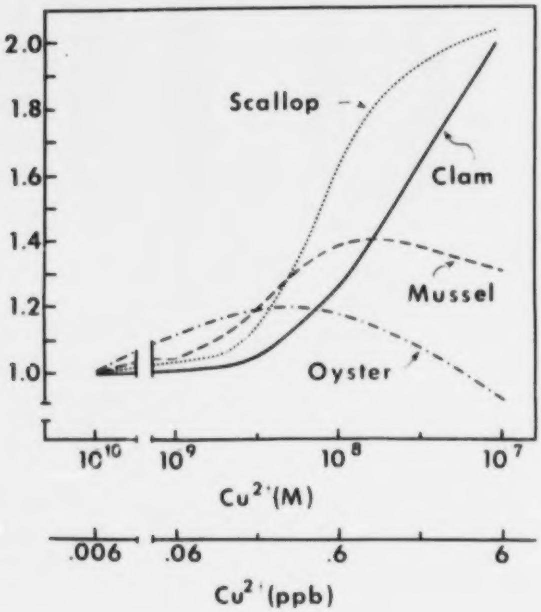

EXHIBIT 3: NORMALIZED RESPIRATION RATES OF MOLLUSCAN GILL TISSUE AS A FUNCTION OF CUPRIC ION CONCENTRATION

In another series of experiments, we exposed gills from clams and oysters to two different salinities and two copper concentrations and recorded respiration rates. The salinities used were 30 ppt and 10 ppt and cupric ion concentrations were $<10^{-10}$M for the controls and $10^{-8.0}$M for the experimentals. Again, the respiration rate was measured with a Gilson respirometer.

The respiration rates of excised clam and oyster gill were affected differentially by salinity and cupric ion (see Exhibit 4). Although salinity had a significant effect upon the respiration of clam gill, it had no effect upon oyster gill. The data on the effects of salinity on gill respiration agree favorably with that of Van Winkle et al. (1968), who demonstrated similar relationships with oyster and clam gill.

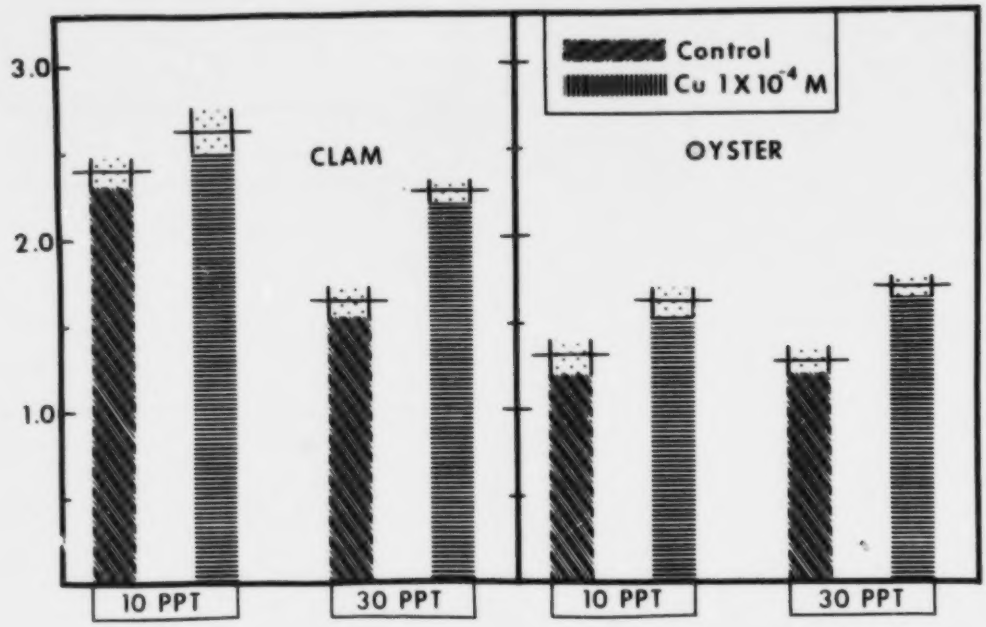

EXHIBIT 4: HISTOGRAM OF CLAM AND OYSTER GILL TISSUE RESPIRATION RATES AS A FUNCTION OF SALINITY AND TOTAL COPPER CONCENTRATION (Cupric ion concentration 10^{-8}M). CONTROL COPPER CONCENTRATION WAS $<10^{-9}$M (Cupric ion concentration $<10^{-11}$M). (Dotted area above and below horizontal line is one standard error.)

On the other hand, copper influenced gill tissue respiration in both species. An ANOVA of the clam data revealed that, while salinity and copper both influenced respiration, there was no significant interaction between the two factors ($\delta = 0.05$). Although the interaction was not significant, it approached significance and suggests that the influence of copper was greater under high salinity conditions. The ANOVA of the oyster gill respiration data demonstrated that the effect of copper on gill respiration was significant, and the increased respiration was not dependent upon salinity (i.e., there was no salinity x copper interaction). The disparity between these data on oyster gill and those from the previous investigation may have been the result of using smaller gill tissue samples (i.e., 50%), and reduced variability in the data. Furthermore, the second experiment was run in a different season of the year.

Because experiments with acute exposures of excised gill tissue to different cupric ion concentrations have demonstrated that cupric ion or copper in the exposure media affects the respiration of excised molluscan gill tissue, experiments were conducted to determine if accumulated copper would significantly affect gill respiration. Oysters were used because of our experience in holding them during extended exposures to metals. We designed a series of experiments to test the effects of accumulated copper upon the respiration rate of oyster gill tissue. The oysters were exposed in a flowing sea water system containing copper at 0.1 ppm as copper chloride; the exposure period was 14 days, with samples taken at 1, 3, 7, and 14 days. After the animals were removed from the exposure tanks, they were allowed to flush in clear water for 24 hr prior to dissection. Tissue was sampled for measurement of respiration, determination of total copper, and fixed for electron microscopy at day 0, 1, 3, 7, and 14.

The accumulated copper in the oyster gill tissue significantly affected the tissue respiration rate by the fourteenth day (see Exhibit 5). There was a steady increase in the respiration rates and copper concentrations of the treated oyster tissue relative to the control tissue from the first through fourteenth day. While the increase in respiration was significant for the copper-treated oysters, there was no significant change in the control tissue respiration over the same time period. It should be noted that by 14 days the remaining oysters were in poor condition, presumably from accumulated copper. Therefore, the increased respiration may be associated with the lethal effect of the accumulated copper.

In addition, ultrastructural examination of oyster gill epithelial tissue was conducted. Preliminary results indicate that there are some dense inclusions present in the tissue, but, at this time, it is not possible to correlate either the densities or numbers of inclusions with copper exposure. We also plan to conduct elemental analysis of the inclusions with the electron probe.

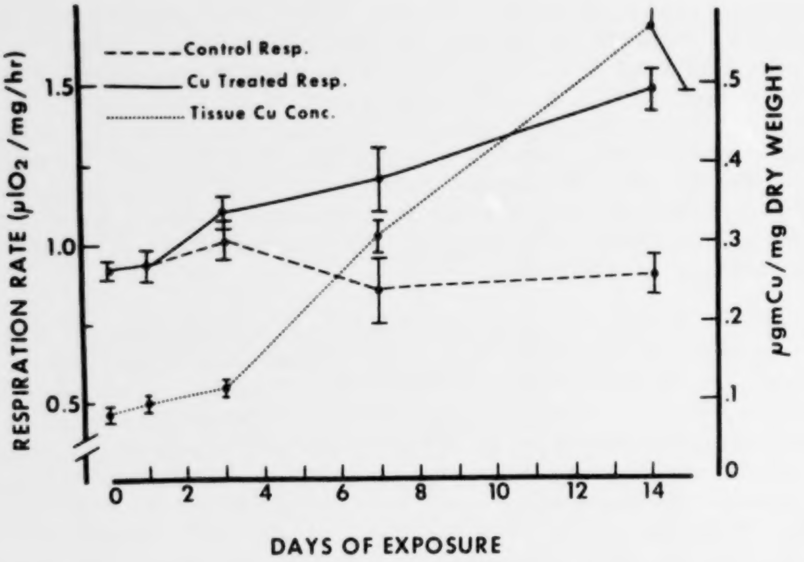

EXHIBIT 5: THE EFFECTS OF TREATMENT WITH 0.1 PPM COPPER ON THE RESPIRATION RATES OF EXCISED OYSTER GILL TISSUE AS A FUNCTION OF TIME OF EXPOSURE, AND THE ACCUMULATION OF COPPER IN GILL TISSUE AS A FUNCTION OF TIME (Vertical bars indicate one standard error of the mean for 10 replicates in the respiration determinations and 5 replicates in the tissue copper concentrations.)

EFFECTS OF CADMIUM ON SHELLFISH

The data on the toxicity of cadmium to aquatic species indicate that fresh water species are somewhat more sensitive to cadmium than marine species (Negilski, 1976; Eisler, 1971), and that there is a salinity dependence of cadmium toxicity on euryhaline organisms (Jones, 1975; von Westernhagen and Dethlefsen, 1975). To demonstrate how salinity affects cadmium toxicity and availability, an investigation was conducted using the grass shrimp, Palaemonetes pugio, as the test organism. To test for toxicity, the shrimp were exposed to 1.0, 0.5, 0.25, 0.125 ppm and <0.1 ppb of cadmium at salinities of 4, 8, 16, and 28 ^{o}/oo. Exposures were made in 4 ℓ polyethylene tubs for 96 hr.

Measurements of cadmium availability and uptake were made using ^{115m}Cd at salinities similar to those used for the toxicity tests. Using liquid scintillation counting techniques, we measured the uptake of the radionuclide by grass shrimp every 24 hr for a period of 96 hr.

The results of the cadmium exposures indicated that the toxicity of the metal was salinity dependent (see Exhibit 6). The greatest toxicity was at the lowest salinity, which indicates that osmoregulatory damage may be involved. Similar data have been generated for marine and estuarine isopods (Jones, 1975) and for marine fish eggs (von Westernhagen et al., 1974). Thus, the salinity dependence of cadmium toxicity appears to be rather universal.

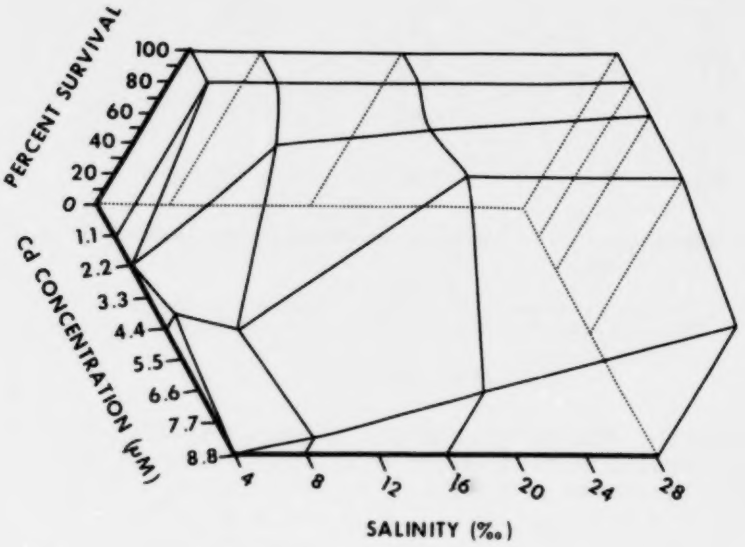

EXHIBIT 6: A THREE-DIMENSIONAL PLOT OF GRASS SHRIMP 96 HR SURVIVAL AT FOUR DIFFERENT SALINITIES AND FIVE DIFFERENT CADMIUM CONCENTRATIONS (Twenty shrimp were used in each treatment.)

When the experiment was repeated and survival of shrimp plotted against cadmium ion concentration rather than total cadmium concentration, the direct correlation between survival and salinity was modified (see Exhibit 7). Survival was dependent upon measured cadmium activity, which, in turn, was dependent upon salinity. The observed change in cadmium toxicity with salinity can be explained through the research of Sunda and coworkers in our laboratory, who have demonstrated that cadmium toxicity is dependent upon the chloride concentration of the water (i.e., salinity), which is from the inorganic complexation of cadmium by chloride. The cadmium-chloride complex is independent of pH and is not affected by dissolved organic matter in the water. Thus, the cadmium-chloride complex is less toxic to grass shrimp than free ionic cadmium.

The uptake curves generated in these experiments also indicated that the bioavailability of cadmium is controlled by the chloride concentration of the water (see Exhibit 8). For shrimp maintained at 5, 10, and 20 ^{o}/oo, the uptake curves were roughly linear for 96 hr, while the data at 30 ^{o}/oo were somewhat more erratic. The rate of uptake was greatest at 5 ^{o}/oo and slowest at 20 and 30 ^{o}/oo. The fact that the

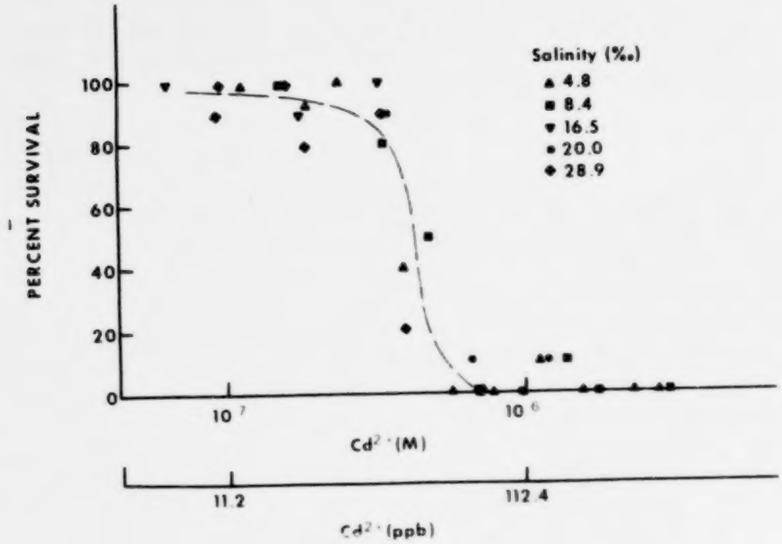

EXHIBIT 7: THE 96 HR SURVIVAL OF GRASS SHRIMP AS A FUNCTION OF THE MEASURED FREE CADMIUM ION CONCENTRATION IN THE MEDIUM

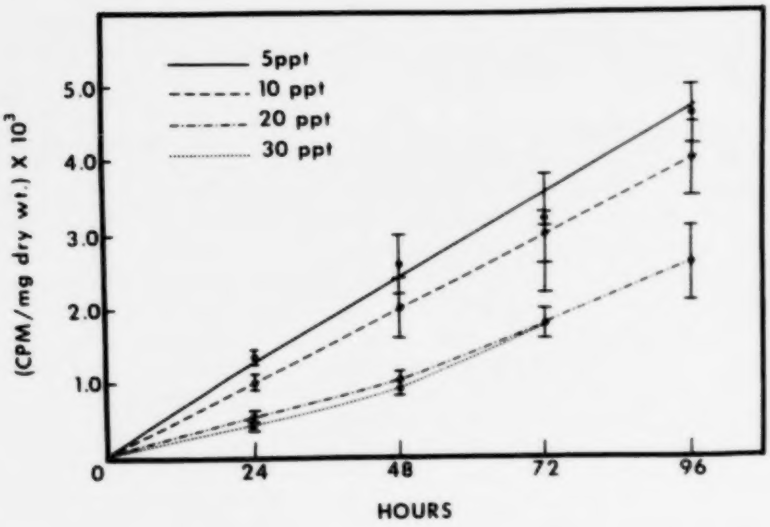

EXHIBIT 8: THE UPTAKE OF ^{115m}Cd BY GRASS SHRIMP AT FOUR DIFFERENT SALINITIES FOR 96 HR (Each point is the mean of five individuals with one standard error.)

curves did not begin to level off by 96 hr simply indicates that equilibrium between the grass shrimp and ^{115m}Cd in the water had not been obtained. To determine if salinity also affects cadmium loss, experiments are planned to test the rate of loss of ^{115m}Cd from shrimp that have been exposed to cadmium at different salinities.

We attempted to measure the effects of cadmium on molluscan gill tissue (i.e., clams and oysters) in a series of experiments similar to the copper series (i.e., both acute exposure and accumulated metal). All experiments were conducted at 30 °/oo and at a series of concentrations of cadmium ranging from 1.0 ppm to 0.125 ppm, but the results were negative. The lack of any effect on the respiration rates of molluscan gill tissue is probably a result of the complexation of cadmium by chloride, since all the experiments were carried out at 30 to 35 °/oo salinity. We can speculate that the complexation of cadmium at high salinity makes it improbable that much, if any, cadmium could pass across a cell membrane. At lower salinities, 15 °/oo and below, our data indicate that this does not appear to be true, and we are currently conducting experiments to test the hypothesis.

The data presented in this report, along with corollary information currently being generated in our laboratory, indicate that the chemical species of an element determines its pharmacological and toxicological effects on aquatic organisms. This has been demonstrated for two elements, i.e., copper and cadmium. The toxicity of copper to algae and fish eggs and larvae (Sunda and Guillard, 1976; Engel *et al*., 1976) was shown to be related to the "free" cupric ion, and that the complexation of copper is by organic chelators and the degree of complexation is pH dependent. Cadmium, on the other hand, is complexed by chloride, an inorganic ligand, and the degree of complexation is independent of pH and the dissolved organic load in the water (Sunda, unpublished data). Thus, it seems reasonable for us to continue our research on the effects of metals, copper, cadmium, and arsenic, using well-defined chemical systems, so that we will also have defined chemical species. Through the use of defined chemical systems, we may be able to demonstrate the mechanisms of action of metallic pollutants on marine and estuarine shellfish.

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ECOLOGICAL CHARACTERIZATION: AN AID TO DECISION MAKING

Howard D. Tait*

Ecosystem studies by the U.S. Fish and Wildlife Service (FWS) and funded through the "EPA energy program" are now operational in four major coastal areas:

1. Chenier Plain area of southeast Texas and southwest Louisiana
2. Pacific Northwest
3. Sea Island - Carolinian
4. Maine.

In this report, the rationale behind these studies, the progress to date, and the work projected for 1977 are reviewed.

RATIONALE FOR ECOLOGICAL CHARACTERIZATION

Coastal areas, particularly wetlands, estuaries, and flood plains, are among the most important fish and wildlife areas on the continent. They are not only important in terms of the birds, mammals, fish, and shellfish they produce, but also for food, recreation, and refuge for man. Rocky coastal areas also serve as vital habitats for certain marine mammals and seabirds; thus, they command the attention of the FWS.

Unfortunately, unless policy decisions reflect ecological reality, the nearshore and onshore areas will receive the brunt of the ecological impacts of expanding energy programs. This is true, whether it be outer continental shelf (OCS) oil and gas development, tidal power, power plants, superports for supertankers, or geopressure energy extraction. The direct impact and the indirect effects must be considered.

It may be useful to consider some of the decisions and needs for information associated with the OCS oil and gas program.

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There are, of course, concerns about the effects of exploratory and production rigs on marine life. Unless lease areas are carefully selected and operations monitored, there is a potential for damaging bottom fauna through discharges of wastes, drill muds, and cuttings. In addition, there is the obvious hazard of oil spills from tankers and accidents on platforms at sea, and much attention is being given to these impacts of the OCS program. The concern addressed here, however, is the onshore and nearshore effects of development.

If there is a substantial find in one of the OCS frontier areas, there will be a need for support facilities, platform fabrication yards, pipecoating and marshalling areas, pipelines, and tank forms. Refineries, gas-processing plants, liquefied natural gas (LNG) processing plants, the petrochemical industry, and associated community services will follow the development. Most of these facilities require waterfront locations along with dredging and dredge disposal and the filling of wetlands. Platform fabrication yards, for example, may occupy 200-1,000 acres of cleared level land adjacent to navigable waterways. Such a fabrication yard is planned at Cape Charles in Northhampton County, Virginia. Environmental impacts are associated with all these facilities; some can be devastating to fish and wildlife.

The adverse ecological effects of these developments may include: turbidity, eutrophication, pollution with toxic materials, basin shoaling and oxygen depletion, benthic habitat degradation, shoreline alteration, saltwater intrusion, and wetlands loss. Therefore, it is critically important that decision makers have available early the essential information on fish and wildlife requirements, the resources of the areas, the impacts to be expected, and the options available to minimize environmental problems.

For instance, the state coastal zone management and fish and game departments, Federal Power Commission (FPC), Bureau of Land Management (BLM), EPA, and the U.S. Army Corps of Engineers, to name a few, will want to know where pipelines or pipeline corridors are contemplated. The FWS would like to be in a position to advise on the optimum routes from a fish and wildlife habitat viewpoint. For each alternative route, we would ask: where are the nearshore and marsh ecosystems, and in what way will they be disturbed by the proposed pipeline route? Are endangered species or critical habitat present? Is dredging and spoil disposal involved? Will nesting birds be disturbed during construction? Will wetlands be crossed? Will digging of a pipeline canal allow intrusion of saltwater with consequent vegetational changes that could diminish the food supply, cover, or nesting habitat of some species? Obviously, insight is needed about both the components of the ecosystems involved and how they interact.

Plainly, decisions regarding OCS development can ultimately have a large effect on fish and wildlife habitat. The decision makers need to know the environmental consequences of alternative courses of action.

Recent court and other governmental actions relative to Lower Cook Inlet and Baltimore Canyon lease areas point up the urgent need for ecological and other information if further delays in OCS development are to be avoided.

It was against this background that the Office of Biological Services (OBS) of the FWS decided to initiate an ecological characterization program early in 1975 with EPA energy "pass-through" funds. It seemed to us that, through characterizing the coastal areas, FWS would serve its own interests of protecting habitat and also provide the many decision makers involved in the OCS and other energy development programs with essential ecological information. The problems encountered with OCS oil and gas development are similar to those associated with other industrial activity, housing, marinas, bridges, causeways, bulkheads, and filling of wetlands for highways. Furthermore, the characterization of coastal areas would materially assist state and local planners and industry in reaching informed decisions about other developments.

ECOLOGICAL CHARACTERIZATION DEFINED

The concept of ecological characterization is not very profound or complicated. We do consider it a "common sense" approach to information gathering and synthesis.

One feature of ecological characterization that distinguishes it from conventional surveys, environmental impact studies, and baseline studies is its emphasis on the functional and dynamic features of coastal systems, rather than on the components within the systems. Baseline studies usually attempt to establish a basis for detecting future change in the ecological attributes considered. An ecological characterization, on the other hand, attempts to provide an appreciation of the existing situation and an understanding of ecosystem dynamics.

The OBS is interested in identifying the structure of the biological communities and the values of chemical and physical parameters; more fundamentally, we are examining the functional relationships among the various components. In the characterization, we are attempting to describe energy flows, nutrient cycles, predator-prey relationships, and community succession. We are also directing particular attention to environmental variables that affect the number and distribution of species which are of special interest to man (e.g., commercial, sport, and endangered species), and which are key to the maintenance of ecological stability and productivity.

The objectives of ecological characterization, then, are to:

- Develop an understanding of the ecosystem function for a given area

- Develop a characterization atlas that presents the information on ecosystems in graphic illustrations, maps, narrative sections, and descriptive or conceptual models
- Involve potential users in the design and review of the studies so that the studies will be responsive to their needs
- Identify additional needs for information and recommend needed studies.

The characterization is being undertaken in two phases: (1) development of qualitative information, and (2) a quantitative phase in which predictive models are developed and environmental impact scenarios run. To date, the OBS has been heavily engaged in phase one: the qualitative aspects.

DEVELOPMENT OF QUALITATIVE INFORMATION

The following criteria were considered in selecting study areas: (1) schedule of OCS and other probable energy developments affecting the candidate area; (2) fish and wildlife values, e.g., presence of National Wildlife Refuges, colonially nesting birds, endangered species, wintering areas; (3) potential of pertinent information being available; (4) size of ecological units in relation to probable cost of doing the study; and (5) other ongoing studies.

We selected the Chenier Plain of southwest Louisiana and southeast Texas for our first study area because of the wealth of data available and because of the area's long experience with oil and gas development. In addition, it is particularly rich in fish and wildlife values.

The Chenier Plain study has been underway now for about 8 months, and Energy Resources Corporation (ERCO), the overall contractor, is responsible for developing and managing the studies, habitat mapping, and report preparation (see Exhibit 1). Louisiana State University (LSU) is engaged in ecosystem modeling, data assembly, and analyses of subsystems. Bendix Applied Science and Technology Division is conducting habitat mapping using the MDAS (LANDSAT imagery) system.

Ecological characterization is intended to serve primarily as a resource management tool. Thus, we are involving the management agencies continually during the planning, data collection, initial testing, and final evaluation. In each case, an advisory steering committee has been established to provide ideas and to assist in locating data. Individuals serving on these committees typically are drawn from state fish and game, and coastal zone management offices, universities, BLM, EPA, National Marine Fisheries Service, and field offices of the service.

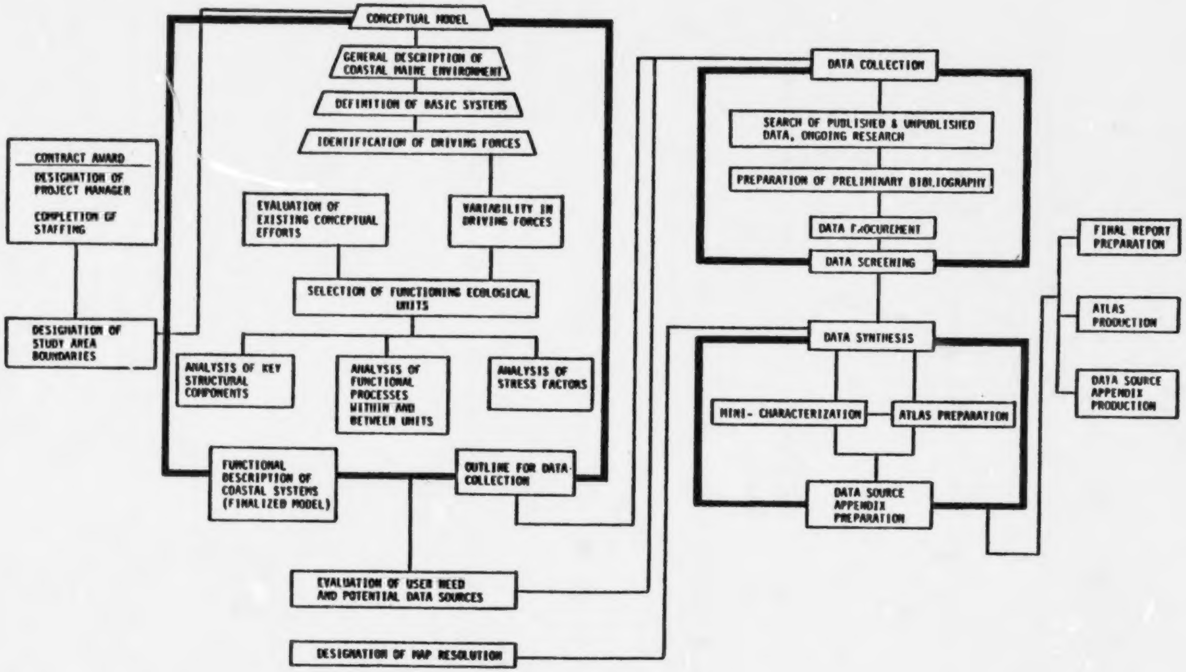
CONTRACT AWARD
DESIGNATION OF PROJECT MANAGER
COMPLETION OF STAFFING
DESIGNATION OF STUDY AREA BOUNDARIES
CONCEPTUAL MODEL
GENERAL DESCRIPTION OF COASTAL MAINE ENVIRONMENT
DEFINITION OF BASIC SYSTEMS
IDENTIFICATION OF DRIVING FORCES
EVALUATION OF EXISTING CONCEPTUAL EFFORTS
VARIABILITY IN DRIVING FORCES
SELECTION OF FUNCTIONING ECOLOGICAL UNITS
ANALYSIS OF KEY STRUCTURAL COMPONENTS
ANALYSIS OF FUNCTIONAL PROCESSES WITHIN AND BETWEEN UNITS
ANALYSIS OF STRESS FACTORS
FUNCTIONAL DESCRIPTION OF COASTAL SYSTEMS (FINALIZED MODEL)
OUTLINE FOR DATA COLLECTION
EVALUATION OF USER NEED AND POTENTIAL DATA SOURCES
DESIGNATION OF MAP RESOLUTION
DATA COLLECTION
SEARCH OF PUBLISHED & UNPUBLISHED DATA, ONGOING RESEARCH
PREPARATION OF PRELIMINARY BIBLIOGRAPHY
DATA PROCUREMENT
DATA SCREENING
DATA SYNTHESIS
MINI- CHARACTERIZATION
ATLAS PREPARATION
DATA SOURCE APPENDIX PREPARATION
FINAL REPORT PREPARATION
ATLAS PRODUCTION
DATA SOURCE APPENDIX PRODUCTION

EXHIBIT 1: APPROACH TO CHARACTERIZING THE STUDY AREA

In general, each study involves the following tasks:

- Definition of the boundaries of the study area along with an initial canvass of available data
- Literature search
- Conceptual model development
- Definition of user needs, usually through questionnaires and interviews
- Designation of map scales and resolution
- Data collection
- Characterization of a pilot area
- Evaluation and revision of methodology based on user evaluation of pilot study
- Preparation of characterization atlas of entire area
- Preparation of data source appendix
- Summary of project outputs.

Exhibit 2 presents the status of these studies. When these tasks are completed, the FWS will conduct a series of user-oriented seminars to help ensure that the utility of the products is understood and that the availability of the reports is known.

EXHIBIT 2: STATUS OF STUDIES

Coastal Area	Date Started	Contractor
Chenier Plain	4/5/76	Energy Resources Co., Inc. Cambridge, Massachusetts
		Center for Wetland Resources Louisiana State University Baton Rouge, Louisiana
		Bendix Applied Science and Technology Ann Arbor, Michigan
Sea Island	2/23/77	South Carolina Wildlife and Marine Resources Department Marine Resources Division Charleston, South Carolina
Maine	2/23/77	Energy Resources Co., Inc. Cambridge, Massachusetts
		Bigelow Laboratory McKown Point, Maine
		Bendix Applied Science and Technology Ann Arbor, Michigan
Pacific Northwest	2/7/77	Ryckman/Edgerley/Tomlinson and Associates, Inc. (Division of Envirodyne Engineers, Inc.) St. Louis, Missouri

FUTURE PROPOSED STUDIES

The studies currently underway will identify gaps in information needed to more adequately understand the ecosystems and to provide the data needed by management. On a case-by-case basis, we will evaluate the practicality of conducting Phase two. This phase involves mathematical modeling to attempt prediction of specific impacts, given certain scenarios. We will also improve the data base with a view to developing digitized, geobased inputs for computer modeling and mapping.

Numerous coastal areas have been proposed for ecological characterization. We are conducting preliminary planning for the following areas:

- Yukon Delta, a vitally important waterfowl-producing area of Alaska
- Lake St. Clair, a water route for fuel transport and an important waterfowl and fishery area
- Eastern Gulf of Mexico (Louisiana, Mississippi, Florida), a site of potential geopressure development, with heavy tanker traffic and superport development
- Southern California, expanding OCS development

- Mid-Atlantic, preferred OCS area for resource potential and heavy tanker traffic

- Pacific Islands (Hawaii, American Samoa and Trust Territories).

A recently completed summary of state information needs prepared jointly by the Office of Coastal Zone Management, National Oceanographic and Atmospheric Administration, and the BLM confirms our belief that ecological characterization studies should be continued and expedited if possible. The following is quoted from page 12 of the January 1977 report.

> "Highest Priority"
>
> The greatest needs for information relate to the potential effect of oil spills on the nearshore environment, the location and impacts of pipelines and onshore facilities, OCS-related employment and the fiscal impacts upon local communities."

AN ECOLOGICAL CHARACTERIZATION OF THE CHENIER PLAIN OF SOUTHWESTERN LOUISIANA AND SOUTHEASTERN TEXAS: OBJECTIVES AND METHODOLOGIES

Janie Harris*

As the nation continues to move toward increased energy development and industrial expansion, we are relying more and more heavily on the environmental impact statement (EIS) process to guide us in our efforts to minimize adverse ecological impacts. However, the current method of incorporating biological and ecological information into EISs has not proven to be a satisfactory predictive tool. Existing ecological impact assessment methodologies have not adequately addressed the complex chain of biological events associated with human activities. The coastal ecosystems team of the Office of Biological Services of the U.S. Fish and Wildlife Service has recently designed a new approach to the investigation of ecological systems. The purpose of this program is to provide a more systematic procedure for both the examination of biological systems and the prediction of impacts on these systems. This new approach, termed "ecological characterization," is being applied first to the analysis of coastal systems.

Energy Resources Company of Cambridge, Massachusetts, together with their subcontractors, the Louisiana State University, Center for Wetland Resources, and Bendix Applied Science and Technology, Ann Arbor, Michigan, have been selected to conduct the first of these coastal ecological characterizations in the Chenier Plain of southwest Louisiana and southeast Texas.

The distinguishing feature of the characterization, which makes it unique in comparison to conventional biological studies, inventories, and other environmental programs, is its emphasis on the functional and dynamic features of coastal systems. By studying these functional attributes of the system, we hope to come closer not only to understanding the driving forces of the represented ecosystems, but also to understanding how the system as a whole is affected by various resource development and environmental modifications.

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PROGRAM OBJECTIVES

The objectives of the Chenier Plain program as outlined by the U.S. Fish and Wildlife Service are essentially fourfold. The first objective is to develop an understanding of the interactive mechanisms and driving forces that govern the area's ecological dynamics. That is, we are interested not only in identifying the structure of the biological communities and the values of various associated physical and chemical parameters but, more fundamentally, we are examining the functional relationships among the various biological and nonbiological system components. These "functional aspects" include energy and material flows, nutrient cycles, predator/prey relationships, species competition, breeding requirements, community succession, and the like. In connection with this effort, we are directing particular attention to environmental variables that affect the number and distribution of the indigenous species which are of special interest to man (e.g., commercial, sport, and endangered species) and those which are key to the maintenance of ecological stability and productivity.

The second objective is to develop a characterization atlas that presents these structural and dynamic features clearly in graphic illustrations, maps, reports, and descriptive models for use by local resource managers and environmental planners.

The third objective is to specify additional information that may be required to characterize the Chenier Plain more completely and to recommend special field studies to generate these data.

The fourth objective is to design an effective and expeditious ecological characterization methodology for future studies, based on our experience gained during the Chenier Plain program.

However, the achievement of these objectives through the performance of the characterization program is not enough, in itself, to change the current methods of ecological impact prediction. Implicit in the development of a characterization program is the need to work closely with the local resource management community and to enlist their suggestions and technical support. Without the enthusiastic support of this community, the characterization program products are unlikely to be used in planning, impact prediction, and resource management efforts. Thus, we are maintaining close communication with the local experts and managers and are soliciting their guidance, criticism, and suggestions so that the final products will, in fact, be responsive to their needs.

This discussion of the Chenier Plain program will address three major topics:

1. Brief overview of the Chenier Plain coastal system

2. Presentation of our basic methodological approach to the characterization effort

3. Description of the outputs that we are producing and an explanation of their utility to investigators and resource managers.

CHENIER PLAIN GENERAL DESCRIPTION

The Chenier Plain, as defined for the purposes of our study, is that coastal area westward of Vermilion Bay, Louisiana, and eastward of Galveston Bay, Texas. It is bordered on the north roughly by the 5 ft inland elevation line and on the south by the Gulf of Mexico 30 ft depth contour (see Exhibit 1).

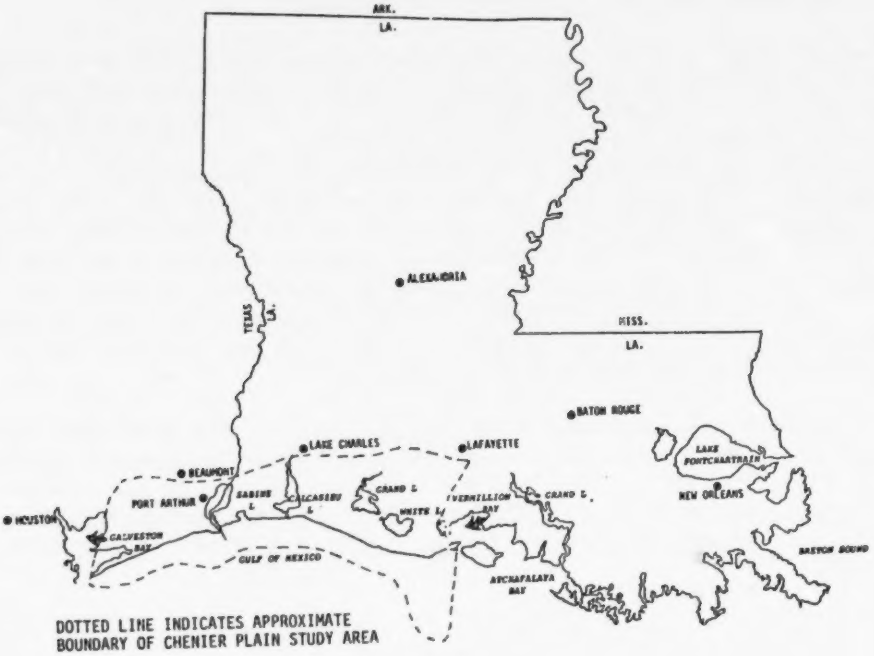

EXHIBIT 1: CHENIER PLAIN STUDY AREA

This area is predominantly a marsh and open water system, demonstrating virtually no relief features aside from the characteristic Chenier ridges, which run roughly parallel to the shoreline. The ridges, which run from a few inches to more than 10 ft in elevation, are approximately 100-1500 ft wide and may extend along the coastline for distances of up to 30 miles.

The presence of these cheniers, which decrease in size and number as one moves westward through the study area, raises some interesting questions relative to the plain's geological history. The Chenier Plain exists as a result of natural accretion of Mississippi River sediments. However, unlike the Deltaic Plain, which grew from the direct deposition of the Mississippi's sediments, the depositional processes contributing to the formation of the Chenier Plain involved sediment transported westward by prevailing longshore currents. These sediments were then deposited along the shoreline as mud flats. These accretionary mud flat areas were then colonized by emergent vegetation, which stabilized the flats and contributed to the continual shoreline progradation and the establishment of a vast marsh system.

Numerous times during the area's geologic history, the Mississippi River shifted its depositional centers. During westward discharges, the plain continued to prograde as described above. During easterly shifts, however, the sediment supply was decreased and accretion slowed or stopped completely, resulting in coastal retreat, as subsidence exceeded sedimentation. This shoreline retreat was coupled with the sorting of shell fragments and sands from the finer clays and silts by wave action. This process resulted in the formation of beaches overlying the mud flat and marsh areas. As the sediment source was renewed by a subsequent shift back to a westerly Mississippi River discharge pattern, the mud flats and marshes would again prograde seaward of the beaches, which then became stranded inland as Chenier Ridges.

The cheniers are very important to the determination of this area's ecological processes, hydrological regime, and human population distributions. They provide pastureland and access routes into the marsh areas for terrestrial species. They are suitable for colonization by large trees, particularly oaks, thus providing a habitat for various bird species.

The Chenier Plain area is characterized primarily by various types of marsh and aquatic ecosystems. These wetland areas are rich in renewable and nonrenewable resources and are subjected to a wide variety of land use practices, most of which involve water level regulation. The major land uses, which direct these management schemes, include oil and gas production; rice, soybean, and crawfish farming; fish and shellfish harvesting; fur trapping; and alligator and waterfowl hunting; as well as maintenance of wildlife refuges and waterfowl preserves. Thus, the employed management strategies are designed to maximize the productivity of the Chenier Plain with respect to these resources.

METHODOLOGY

The first phase of the characterization program involves a qualitative assessment of the major functional interactions within the Chenier Plain system. In the second phase, we plan to employ a variety of analytical techniques to quantify the ecological relationships and energy and material flows. The second phase, then, will allow us to develop predictive models and to run environmental impact scenarios. Because the second phase has not yet been initiated, this discussion will be restricted to the methodology and products of the first phase, which involves six separate tasks:

1. Development of a conceptual model and the determination of data needs
2. Data collection/data synthesis
3. Production of a working habitat map
4. Pilot characterization and methodology evaluation
5. Final characterization
6. Production of characterization atlas and reports.

The conceptual model, which is the guiding element of the characterization, is essentially a portrayal of the principal ecosystem components and fundamental relationships that link them. By identifying the major features and interactions, this model provides the focus for the requisite analysis as well as the framework for data collection.

The first step in conceptual model development was to divide the Chenier Plain into a set of subsystems, each of which is internally homogeneous with respect to certain key variables. This subdivision was performed on three levels of complexity as based on six key environmental variables: water flow patterns, elevation, vegetation type, inundation and salinity regime, and man-imposed environmental modifications.

The three levels of complexity are: the regional level, the drainage basin level, and the habitat level. At the broadest level, the model treats the geologic history and geomorphological features of the entire area. At the intermediate level, the drainage basin, the model identifies six distinct hydrologic units and addresses the ecological interactions among the various habitat types within each basin. We believe that the interhabitat interactions defined at this level are largely governed by the water flow patterns, inundation characteristics, and salinity regime. The habitat level of the conceptual model addresses the major community assemblages that characterize the plain and provides the framework for defining and investigating the functional relationships among the physical, chemical, biological, and ecological components within the 14 major habitats identified in the Chenier Plain.

These 14 habitats are identified as:

Aquatic Habitats	1.	Inland open water systems, including both lakes and bayous
	2.	Nearshore gulf waters
	2a.	Mudflats
Wetland Habitats	3.	Salt marshes (including small ponds)
	4.	Brackish marshes (including small ponds)
	5.	Intermediate marshes (including small ponds)
	6.	Fresh marshes (including small ponds)
	7.	Swamp forests
Upland Habitats	8.	Upland forest (including pine forest)
	9.	Ridges (cheniers, artificial and natural levees, and spoil banks)
	10.	Beaches
Altered Habitats	11.	Rice fields and other managed crop areas
	12.	Pasture lands and grassland prairies
	13.	Managed marshes (for waterfowl, etc.)
	14.	Urban areas and industrial sites.

For each of the 14 components of the habitat level of the model, a schematic diagram was developed to present the major components and the interactions occurring among them. This schematic is supplemented by an interaction matrix that specifies the nature, direction, and variability of the important interactions.

Once the key features and interactions were specified for each of these abstracted habitats types, we mapped the distribution of the habitats as they occur in the Chenier Plain. This mapping allowed us to aggregate the available data sets by habitat type and to investigate and compare the functional interactions that characterize similar and dissimilar systems.

Our procedure for generating this working habitat map involved the processing of LANDSAT satellite imagery of the area. The LANDSAT system distinguishes different habitat types based on their reflective properties. A specific area of the Chenier Plain was identified as a particular habitat type by a ground truth expert. The automated LANDSAT system then identified all areas that have the same reflective properties as the sample area and assigned them a particular color code. The habitat map was then refined by adding information from aerial photographs, existing maps, literature, and ground truth to produce a sufficiently detailed map.

The next step in the program is the performance of a pilot characterization for a portion of the total study area. This task enables us to test our conceptual model and characterization methodology on a small area and to generate a pilot study atlas to review selected display techniques. Thus, we can evaluate our approach and refine it before the characterization of the entire area is undertaken.

PROJECT OUTPUTS

At the conclusion of the first phase of the Chenier program, we will have six major outputs that will be available through the U.S. Fish and Wildlife Service. These products include: the finalized conceptual model document, the characterization atlas, a bibliography of relevant information sources, a computerized data base, a report recommending field studies that will help fill crucial data gaps, and a set of methodological recommendations to guide future characterization efforts.

Clearly, the characterization atlas, the bibliography, and the computerized data base will most directly address the information needs of investigators, resource managers, and people responsible for the preparation of EISs. The conceptual model, field study recommendations, and methodology outline are being prepared primarily for use by investigators involved in future characterization studies and those who update the ongoing studies.

Hopefully, the characterization approach to the examination of ecological systems, by focusing on the dynamic and functional aspects of the system, will help lead scientists away from the temptation to describe ecosystems as if they were static in time and space. Our ultimate objective, then, is to present the Chenier Plain coastal system as a set of functionally interactive subsystems, defined in such a way that impacts on important biological communities can be predicted and evaluated in the context of the basic interacting forces which define the system _in toto_. Moreover, many of the elements of the characterization effort are important not only because they allow us to understand the primary and secondary environmental modifications expected to result from a proposed resource development but also because they bring us closer to the understanding of the composite system and how it functions.

DISCUSSION

Dr. Lefcort: The Fish and Wildlife Service is supporting a contract with the Conservation Foundation to assess the onshore impact of offshore development. In this computerized data retrieval system, you query the computer and you get the answer in terms of what is available, everything you have always wanted to know.

In terms of OCS development, these two projects are quite similar. Where are the areas of overlap?

Dr. Tait: They are not overlapping. They are very complementary. The computerized aspect of the Conservation Foundation Project is still in an experimental stage. It has a lot of promise because there is a lot of data that can be fed into that system: the quantitative data from these characterizations; information on endangered species and their habitats; and the material that the Conservation Foundation itself will generate relative to onshore impacts.

Dr. Palmisano: The Conservation Foundation contract is kind of a cookbook of how to put together all of the complex activities associated with OCS development. It attempts to present a typical sequence of events related to OCS, beginning with the leasing all the way through to development, and put this information in a time and space perspective. Associated with that is an analysis of the impacts of these activities on resources. This model can be applied anywhere in the United States, on any coast, under any situation. However, you have to go to a specific area, such as the Chenier Plains, and look at the biology and ecology of that particular area, and apply the guidance in the Conservation Foundation Guidebook.

It first outlines a broad program, OCS, say, for mid-Atlantic. It then describes the project, which is a broad activity that probably will require an impact statement such as the location of a nonshore facility storage tank facility. This project will have a subproject, such as a navigation channel and a subproject has an activity. An activity is the level at which permits are usually issued, for dredging, bulkheading, land filling, and so forth. In this way you can look across an entire OCS development program.

Dr. Barrientos: Will you get storm search data?

Dr. Tait: I think any storm search data that we have will be that immediately available from other records.

THE CONSTRUCTION OF A CONCEPTUAL MODEL OF THE CHENIER PLAIN COASTAL ECOSYSTEM IN TEXAS AND LOUISIANA

L.M. Bahr, Jr.,* J.W. Day, Jr.,* T. Gayle,† J.G. Gosselink,*
C.S. Hopkinson,* D. Stellar,* and J. Harris**

Increasing interest in coastal areas on the part of environmentalists, developers, and managers has generated the need to understand the function of productive and fragile areas and to predict the effects of further alterations to them. The term "function," as used throughout the following description of the Chenier Plain conceptual model, is intended to describe the mechanics of the ecosystem, i.e., the pathways and processes by which energy and matter is captured, transferred, partitioned, stored, cycled, and degraded by the system. Examples of functional processes include primary production, water flow, trophic exchanges, and animal migrations. A functional understanding of an ecosystem includes much more than an inventory of important physical parameters and organisms; it requires a holistic, systems-level analysis to identify all important interactions among biological and physical components of the system and all important control features and feedback mechanisms.

The U.S. Fish and Wildlife Service (Department of the Interior) decided in late 1975 to fund an exhaustive study of the Chenier Plain coastal ecosystem(s) of Southeastern Texas and Southwestern Louisiana (Galveston Bay, Texas, to Vermilion Bay, Louisiana), in which the area would be characterized ecologically by the development of a conceptual model of the system and a synthesis of all extant data. This characterization was designed to serve as a pilot study for future projects that will eventually describe all U.S. coastal ecosystems. The specific request was for a "description of the important resources and processes comprising the ecosystem and an understanding of their functional relationships." The first requirement of this study (and the key to

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the entire project) was the formulation of a conceptual model of the ecosystem(s). The model was to consist of a schematic framework of ecosystem function in which all important processes and interactions among components would be identified in a qualitative manner. The completed model would identify data requirements and gaps, and set the stage for the two remaining portions of the study, a characterization atlas, and a quantitative ecological simulation model of the study area that could be used to aid in making management decisions.

DESCRIPTION OF THE PROBLEM

Any ecological model of the Louisiana-Texas Chenier Plain must take into account at least the following four factors:

1. Spatial heterogeneity. The area described as the Chenier Plain (see Exhibit 1) is highly variable in space; from east to west, it is broken up by a series of rivers flowing southward into the Gulf, through lakes of different sizes and salinities, and over thousands of square miles of wetland. The wetlands themselves are not at all homogeneous; vegetation ranges from pure stands of saline oyster grass (*Spartina alterniflora*) to fresh water bull tongue (*Sagittaria falcata*) and maidencane (*Panicum hemitomum*). They are cut by elevated cheniers or ridges, which function ecologically in a very different manner than the adjacent wetlands. Large areas, both wetlands and highlands, have been modified for agriculture or are managed for waterfowl or furbearers. The region is far from homogeneous, and any model that ignores this will produce information of limited management value.

2. Ecological or functional complexity. Aside from this spatial heterogeneity, the ecological food web is enormously complex within any small, fairly homogeneous area. On the whole, it is poorly understood.

3. Time scale of events. Events of ecological interest in the Chenier Plain occur at the scale of hours, days, or seasons for many biological processes; years or tens of years for many cumulative human impacts, such as canal dredging and eutrophication; and thousands of years for geological processes that determine the physiography of the whole region. It is difficult to visualize a useful model that can simultaneously simulate geological processes and microbial kinetics in terms that are useful to a manager.

4. Management needs. In addition to the above considerations, the model must enable a manager to evaluate the consequences of alternate management strategies at appropriate levels of spatial, ecological, and temporal resolution. Existing models cover a wide range of approaches, including strategies to exploit or manage single commercial species, such as fishery models (Wagner, 1969) or

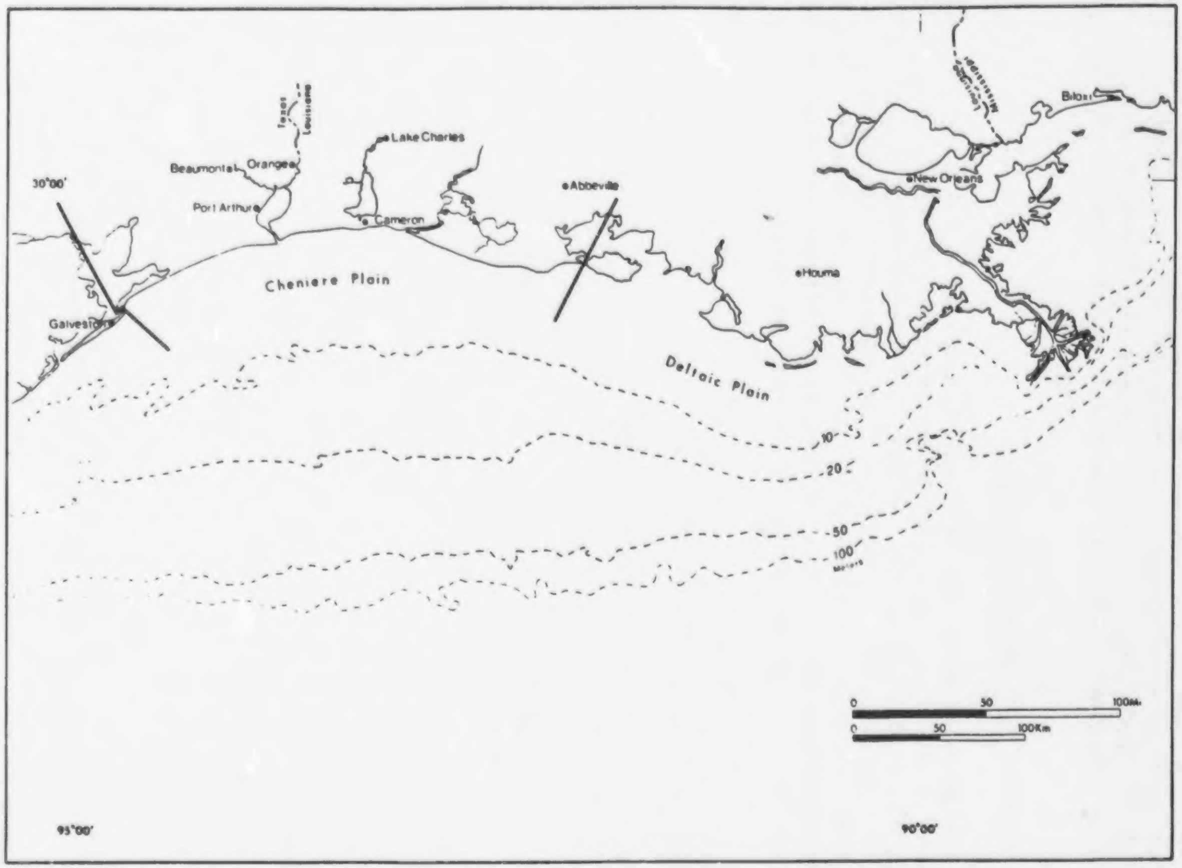

EXHIBIT 1: THE CHENIER PLAIN REGION OF SOUTHWESTERN LOUISIANA AND SOUTHEASTERN TEXAS

alligator models (Nichols et al., 1976); models that treat ecosystems as homogeneous in space in order to elaborate the energetic interactions (Patten et al., 1975; Wiegert et al., 1975); or models that treat spatial heterogeneity but consider only a limited number of chemical or biological parameters (Kremer and Nixon, 1975) and dramatically simplified, dynamic world view models (Forrester, 1971).

THE STRUCTURE OF THE CONCEPTUAL MODEL

In its present form, the conceptual model is a hierarchy of nested models that divide the Plain into three levels of spatial, functional, and temporal resolution or the habitat, the basin, and the region (see Exhibit 2). By focusing attention at the appropriate level, this hierarchy allows the characterization and analysis of the Chenier Plain and the handling of the four issues of spatial heterogeneity, functional complexity, time scale problems, and management needs.

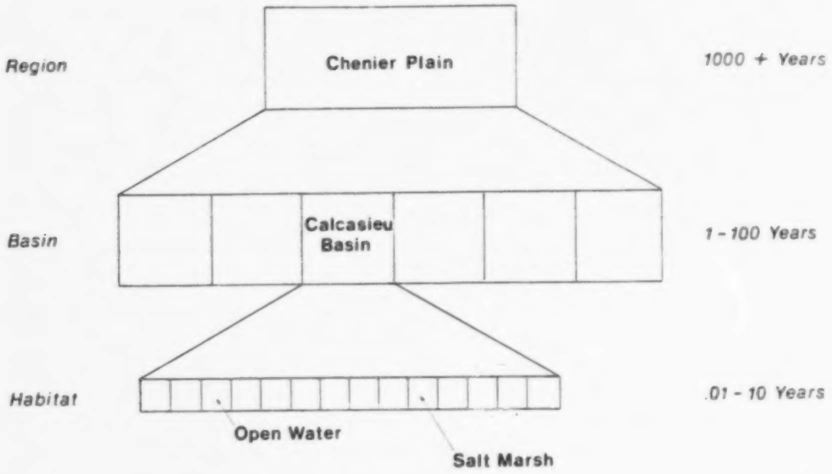

EXHIBIT 2: SCHEMATIC ILLUSTRATING THE TIME SCALE OF EVENTS OF INTEREST FROM HABITAT TO REGION

The smallest identifiable ecological units are called habitats. Wherever a particular habitat occurs on the Chenier Plain, it is recognizable as the same basic functional unit, and can, therefore, be treated as homogeneous, even though we recognize the existence of gradients, specialized niches, and discontinuities within a habitat.*

Each habitat is a complex ecological system. Each is characterized by its own species, carrying capacities for those species, levels of production, food web, nutrient cycles, and physical inputs, wherever it is found. The time scale of important events is often seasonal, and short-term impacts are important at this level. Most habitats are intuitively distinct. For example, aquatic areas are quite different from upland forests; however, different kinds of natural wetlands are not so clearly unique.

For the Chenier Plain, we have identified and mapped 10 natural habitats: nearshore Gulf; inland open water; salt, brackish, intermediate, and fresh marsh; wetland forest; upland forest; beaches; and cheniers and ridges. Large areas have been modified by human activity, which we have catalogued into four additional habitats as impounded marshes, pastures, rice and crop habitat, and urban habitat.

Habitats interact at a broader level of resolution, i.e., the basin. Drainage basins represent perhaps the most natural ecological units of the region, because each basin is integrated by the flow of water over and through it. Six fairly distinct basins have been identified on the Chenier Plain (see Exhibit 3, and Bahr et al., 1977). Each basin has its own hydrodynamic characteristics determined by upstream flow, elevation and slope of the basin, and extent of its connection with the Gulf via tidal passes.

At the basin level, emergent properties such as interhabitat interactions become important, and intrahabitat details are ignored by treating each habitat as a black box with known inputs and outputs. The system is no longer treated as homogeneous in space. Most significant changes occur through large-scale or cumulative impacts over a period of time measured in years, not in days or seasons. Examples include: impacts of deep shipping channels on saltwater intrusion; gradual changes in hydrology associated with stream channelization; continuous canal dredging and associated spoil bank formation; and cumulative wetland drainage, a little at a time, for urban and industrial development.

The six basins of the Chenier Plain are unified by a common geologic history. Geologic processes unify the region and determine the specific characteristics (e.g., slope, relief) of each basin, but for most purposes these characteristics can be considered as unchanging in the time scale of basin or habitat-level phenomena.

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* See Bahr et al. (1977), for justification of habitat concept.

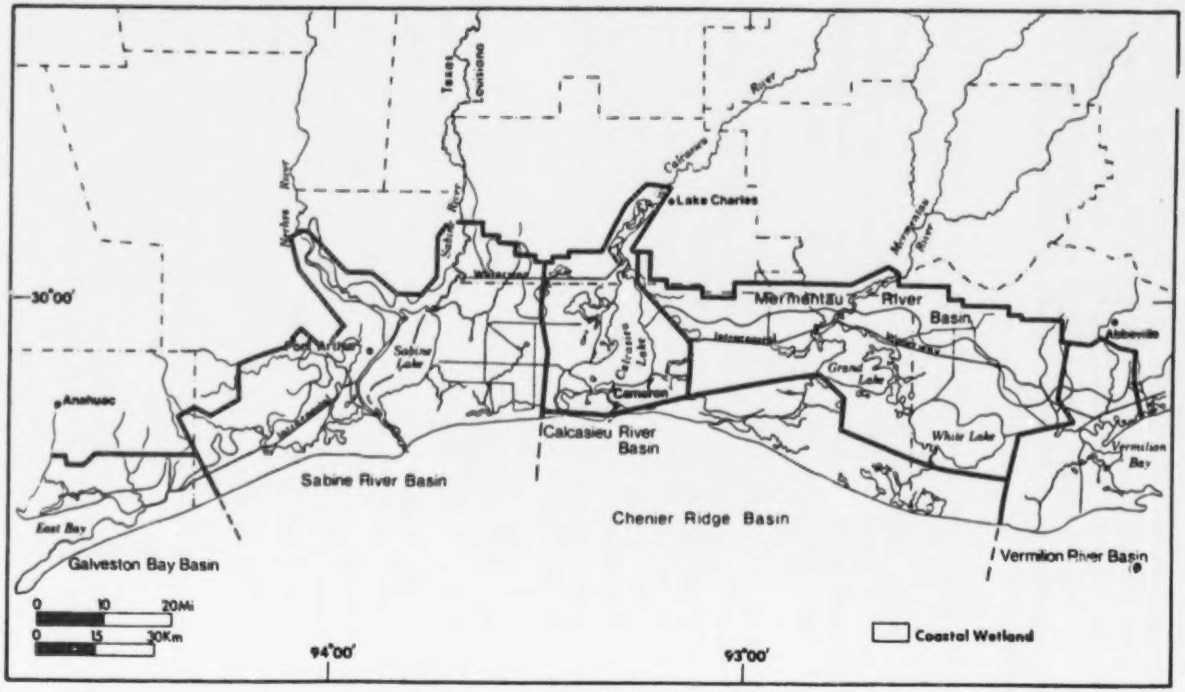

EXHIBIT 3: WATER IS THE PRIMARY INTEGRATING ELEMENT IN THE SIX BASINS IN THE CHENIER PLAIN REGION

We have developed conceptual models at all three levels of the hierarchy, but for the reasons discussed below the emphasis of the quantitative model is at the basin level.

The regional model is primarily a model of geological processes (see Exhibit 4), which are not strongly influenced by man except as he controls the flow of the Mississippi River. Beach and mudflat growth or erosion depends on the interaction of depositional and erosional processes that use and rework longshore and beach sediments. When deposition predominates, mudflats build offshore of the beach. During periods of erosion these mudlfats are reworked and winnowed to leave sand beach ridges. When net deposition occurs again and mudflats build out, the beaches are stranded in inland cheniers.

To give a qualitative functional understanding of the habitat and guide the acquisition of data, complex habitat level models have been drawn for each of 14 habitats. However, quantitative mathematical models at this level are more a subjective art than an objective science (see Exhibits 5-7).

For several practical reasons, we have concluded that major emphasis for management purposes should be placed at the basin level. Development of a functional mathematical model to simulate the full range of ecological complexity of the Chenier Plain would represent a staggering undertaking. Furthermore, when we consider the success of similar models by others and our understanding of basic ecological interactions, such a model would not necessarily be very useful. In addition, both the major kinds of manageable processes and the time scales of manageable events appear to occur at the basin level; changes in area of habitats, such as the gradual increase in agricultural area at the expense of wetland; eutrophication from urban growth; and, perhaps most significant, the gradual cumulative changes in hydrology associated with dredging many small canals over a period of many years.

An additional consideration is our ignorance of the internal workings of a habitat. Thus, we manipulate habitats at some peril to the function of the whole system. A better approach to management is to recognize that certain renewable resources or nonresources (Ehrenfeld, 1976) are associated with each habitat and that to protect the resource one must protect the habitat.

ASSUMPTIONS OF THE BASIN LEVEL MODEL

The basin level model was developed with the following assumptions:

1. A habitat can be treated as a "black box." That is, a habitat normally characterized by certain plant species with a predictable level of primary production. Given this level of primary production, the habitat will have a "natural resource potential" (NRP), which is quantifiable as a yield or carrying capacity for certain

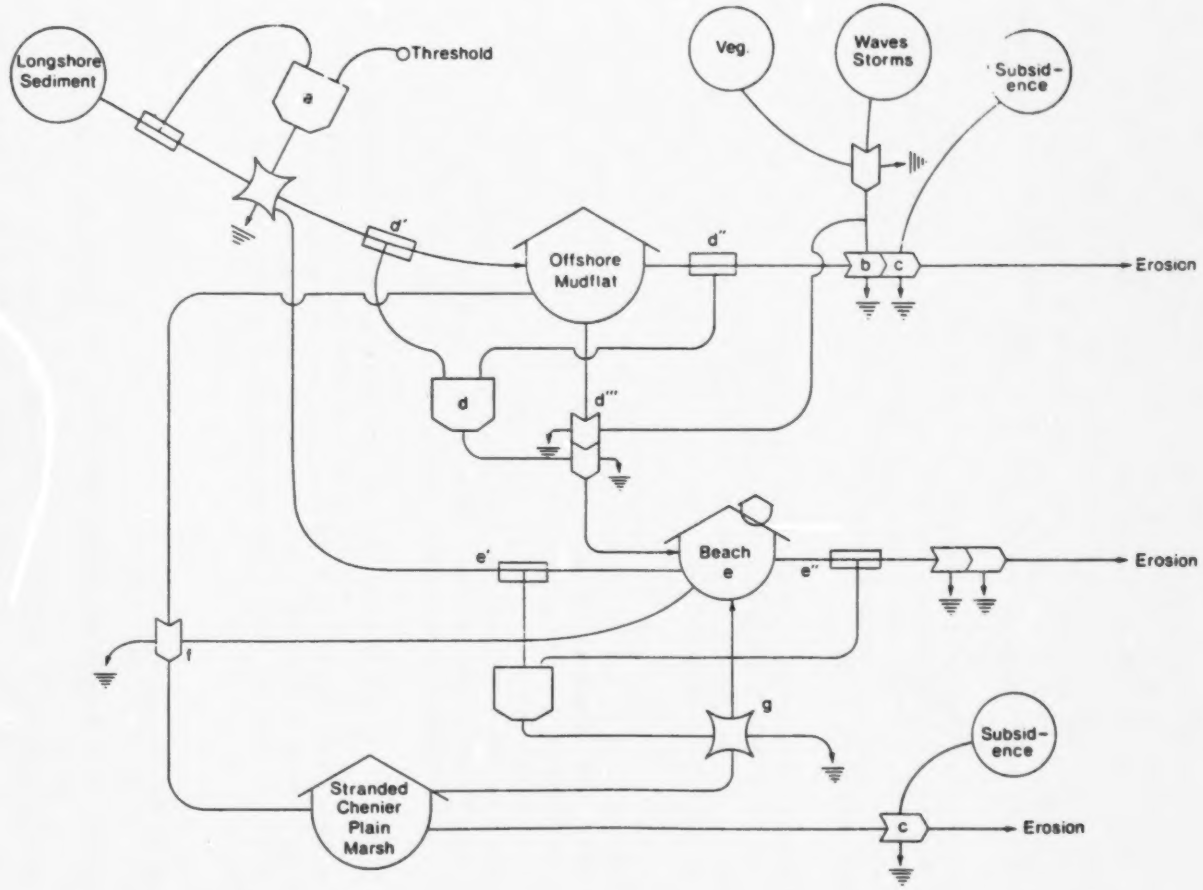

EXHIBIT 4: DIAGRAMMATIC MODEL OF REGION-LEVEL GEOLOGICAL PROCESSES

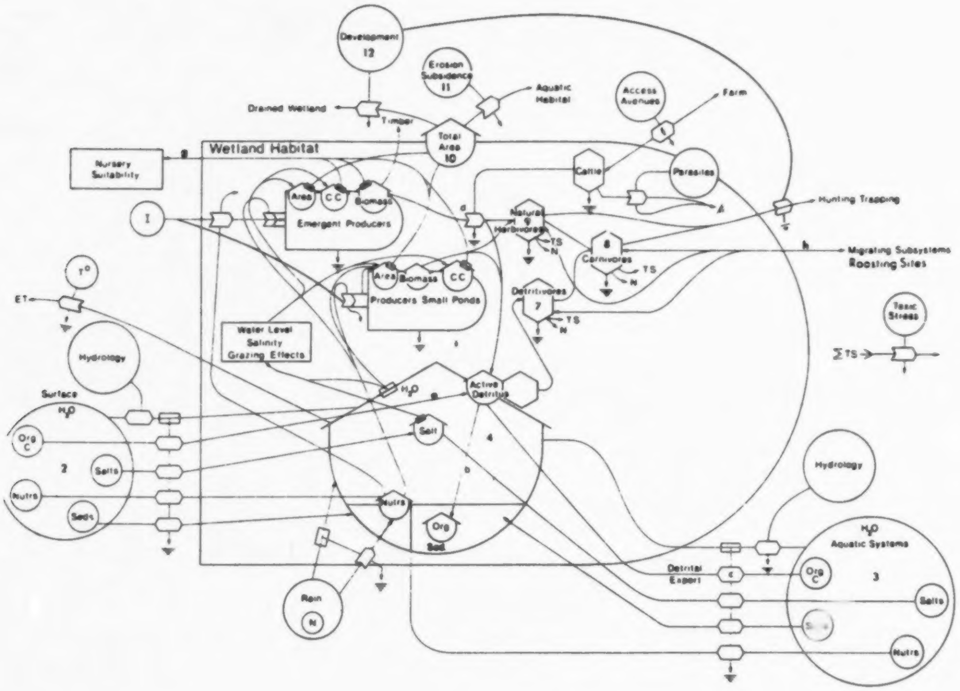

EXHIBIT 5: DIAGRAMMATIC REPRESENTATION OF AQUATIC HABITATS
[The habitat is represented as a water storage module (1), with water flows controlled by upstream and tidal head (2). Water carries nutrients, salts, toxins, and sediments into and out of the habitat. These materials control the species present and productivity of phytoplankton (3), floating aquatics (4), and benthic producers (5), which in turn support consumer groups (6, 7, 8, 9). Organic detritus (10) is an important organic source to the system.]

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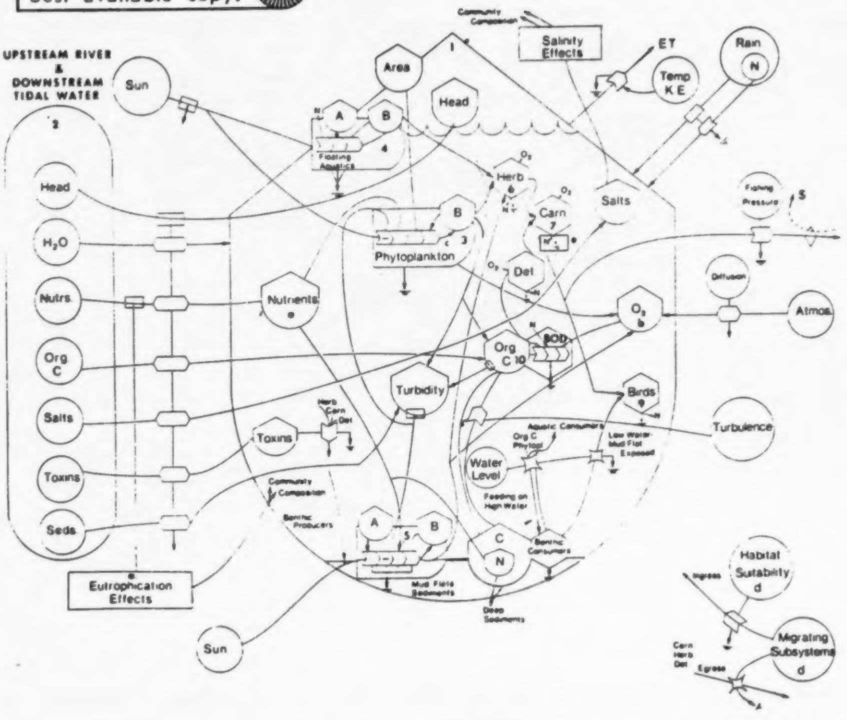


EXHIBIT 6: DIAGRAMMATIC REPRESENTATION OF WETLAND HABITATS [The habitat is shown as a producer (1) that exports organic carbon. Upstream (2) and downstream (3) water flows control the hydrologic regime of the wetland (4), and the flux of organic carbon, nutrients, toxins and sediments. Emergent producers (5) and producers in small ponds (6) feed detritus to consumers (7, 8, 9), which migrate through wetlands, are hunted and trapped. Habitat area (10) is controlled by natural processes (11), and by urban and agricultural development (12).]

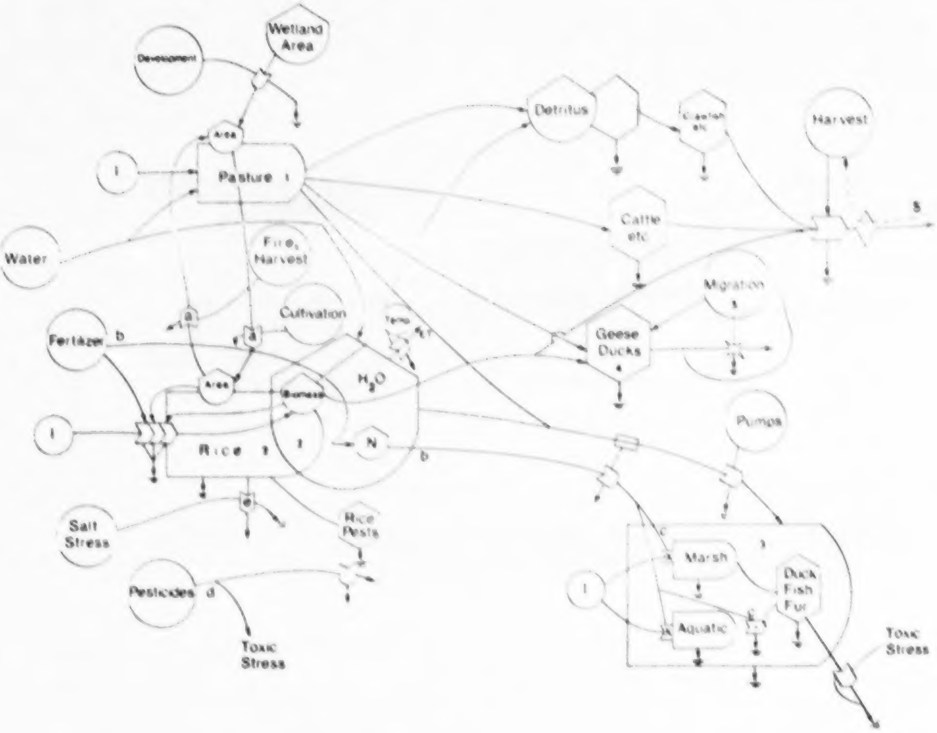


EXHIBIT 7: DIAGRAMMATIC REPRESENTATION OF MANAGED HABITATS, PASTURE (1), RICE (2), AND MANAGED MARSH (3). [Much of this area is maintained by burning, cultivation, draining and/or controlled flooding, at the expense of wetlands. Food chains are simplified, but considerable interaction occurs with adjacent habitats (4, 5).]

species of interest to man (see Exhibit 8). For instance, a fresh marsh will have a high potential for nutria (∿90 pelts/100 acres) and dabbling ducks (peak density, 270 ducks/100 acres) but a rather poor potential for muskrats, which are concentrated in the brackish marsh (∿40 pelts/100 acres).

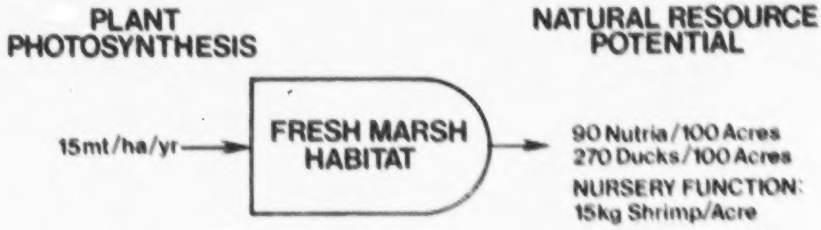
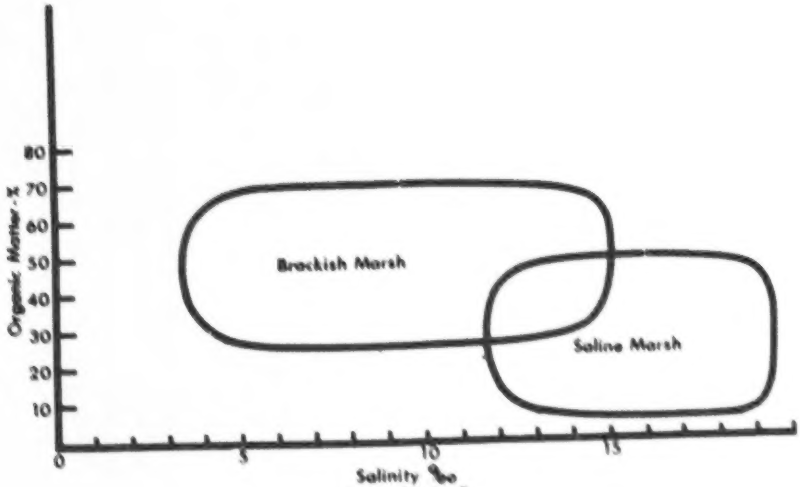


EXHIBIT 8: MATERIAL RESOURCE POTENTIAL OF A HABITAT IS DETERMINED BY LEVEL OF PLANT PRODUCTION

This concept is implied in the notion of "carrying capacity," which is extensively used in wildlife management. Identification of the NRP for a particular habitat does not demand a detailed understanding of the processes that result in carrying capacities for specific organisms. Rather, it is an empirical observation of the value of a certain kind of habitat for certain species.

2. Natural habitats are determined by a set of physical environmental parameters. It is generally felt that ecological succession in wetland habitats is controlled by the physical environment, i.e., allogenic processes dominate over autogenic ones (Odum, 1971). Thus, if the driving (controlling) environmental forces (such as hydrologic regime) change, the habitat will change its identity and become a different habitat. An example of this process is the gradual intrusion of salinity in the Barataria Basin of Louisiana, which is causing a landward shift in the boundary between salt and brackish marsh and increasing the area of salt marsh habitat at the expense of brackish marsh. For wetlands, the major controlling parameters are salinity and the inundation regime (see Exhibit 9).

3. Water is the integrating mechanism of the basin (see Exhibit 10). Many of the interactions among habitats, such as organic and nutrient fluxes, are dependent on water flows. Aquatic consumers move among aquatic and wetland habitats via flooding waters. In



Habitat "niches"

from Chabreck, 1972

EXHIBIT 9: WHEN WETLAND HABITATS ARE PLOTTED ON A GRAPH OF SALINITY OF FLOODING WATERS VS SOIL ORGANIC CONCENTRATION, THE "SPACE" OCCUPIED (MEAN ± SD OF EACH PARAMETER) BY SALT MARSH OVERLAPS BRACKISH MARSH VERY LITTLE

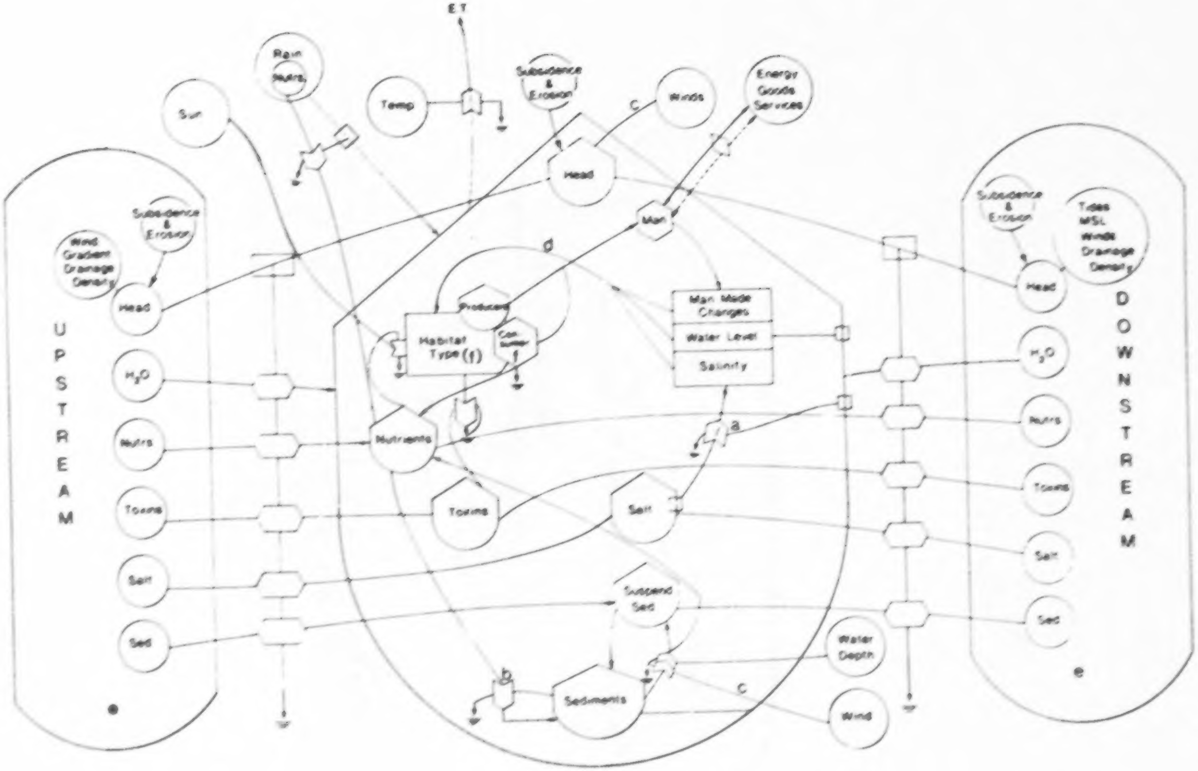


EXHIBIT 10: A DIAGRAMMATIC REPRESENTATION OF A COASTAL BASIN [The basin is represented as a water storage module, in which water (and dissolved and suspended materials) flow is controlled by the upstream and downstream water regime. Habitat area, quality, and interaction are controlled by these flows.]

addition, changes in the pattern of inundation (i.e., frequency, depth, and duration of flooding) mediate changes from one habitat to another.

A major identifiable characteristic of a drainage basin is the change in area of different habitats, constrained by the constant size of the whole basin. Historical rates of change of habitats within a basin can be determined with accuracy from old maps (see Exhibit 11). The change in area of habitat within a basin is controlled by natural and man-related processes. Major shifts are from natural wetland to open water, impounded wetlands, agriculture, and to other developments.

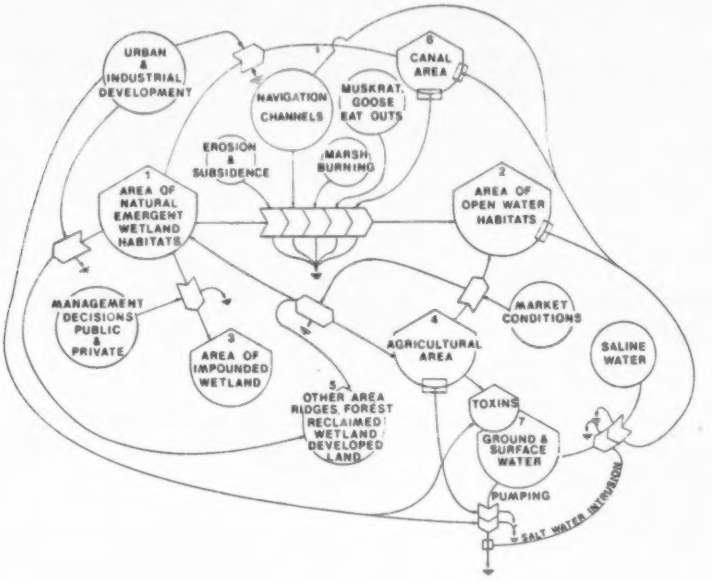

URBAN & INDUSTRIAL DEVELOPMENT
NAVIGATION CHANNELS
MUSKRAT, GOOSE EAT OUTS
6 CANAL AREA
EROSION & SUBSIDENCE
MARSH BURNING
1 AREA OF NATURAL EMERGENT WETLAND HABITATS
2 AREA OF OPEN WATER HABITATS
MANAGEMENT DECISIONS PUBLIC & PRIVATE
3 AREA OF IMPOUNDED WETLAND
MARKET CONDITIONS
4 AGRICULTURAL AREA
SALINE WATER
5 OTHER AREA RIDGES, FOREST RECLAIMED WETLAND DEVELOPED LAND
TOXINS
7 GROUND & SURFACE WATER
PUMPING
SALT WATER INTRUSION

EXHIBIT 11: SCHEMATIC ILLUSTRATING THE CHANGE IN AREA OF HABITAT WITHIN A BASIN

For management purposes, we can project changes in habitat area from our knowledge of hydrologic processes of the basin and of the ways in which modification of these processes changes habitats. In practice, a good two-dimensional hydrodynamic model is necessary to predict hydrologic changes, salinity intrusion, etc., resulting from canal dredging and spoil deposition.*

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* The Louisiana State University Center for Wetland Resources has such a model operating for Barataria Bay, Louisiana, but assessment of hydrologic changes in the Chenier Plain study is still primarily qualitative.

In addition to changes in habitat area, the productivity of a unit area (the resource potential per unit area) can be changed by such processes as eutrophication, introduction of pesticides and other toxins, water level control by weirs and levees, and overharvesting of existing resources (see Exhibit 12).

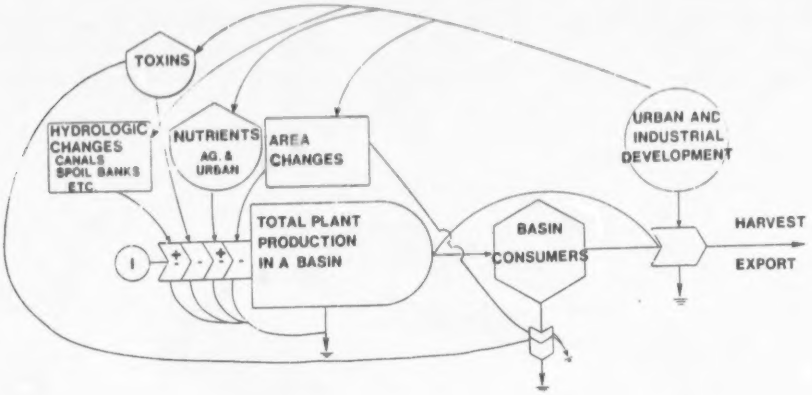

EXHIBIT 12: SCHEMATIC ILLUSTRATING THE CHANGE IN THE TOTAL NATURAL RESOURCE POTENTIAL OF A BASIN

From a knowledge of the current natural resource potential of each habitat in a basin and of areal and qualitative changes in each habitat over time (or because of human intervention), one can project the total change in the basin NRP. This change (which is almost always negative) can then be compared with the expected benefits of the activity to be managed.

As an example, the Calcasieu basin in the Chenier Plain is estimated to support total primary plant production (a rough index of the total value of the natural basin landscape) of 2.3 million metric tons per year. This produces an estimated 1,000 mt of shrimp, 7,700 mt menhaden, 5 million sport fishing efforts, 222,000 dabbling ducks at peak density, and 49,000 fur pelts.

Since 1953, 73 square miles of natural marsh has been lost (Exhibit 13), 70% of it to open water, the rest to impounded marshes, agriculture and urban habitats. This figure represents a loss of 26% of the total natural marsh area of the basin. The consequences in natural resources are estimated very roughly at a loss of 260,000 mt of organic production, 77,000 birds per year, 19,000 fur pelts, and significant impact on the shrimp and menhaden fisheries.

EXHIBIT 13: CHANGE IN AREA OF HABITATS IN THE CALCASIEU, LOUISIANA BASIN BETWEEN 1953 AND 1975

Habitat	Area (sq. miles)		Net Area Gain (Loss)	Net % Change (1953-1975)
	1953	1975		
Open Water	107.52	158.13	50.61	47.1
Nearshore Gulf	156.00	155.38	(0.62)	- 0.4
Natural Marsh	284.30		(72.71)	- 25.6
Saline		8.28		
Brackish		101.66		
Intermediate		78.81		
Fresh		22.84		
Altered Marsh	23.91	37.65	13.74	57.5
Ridge				
Natural	32.45	31.12	(1.33)	- 4.1
Spoil	9.58	12.78	3.20	33.4
Agriculture				
Rice	17.03	22.05	5.02	29.5
Non-rice	5.18	6.01	0.83	16.0
Pasture	24.41	23.05	(1.36)	- 5.6
Urban	3.28	8.79	5.51	168.0
Upland Forest	7.50	5.52	(1.98)	- 26.4
Beach	3.51	3.26	(0.25)	7.1
Swamp Forest	3.42	2.76	(0.66)	- 19.3
TOTAL	678.09	678.09	0.00	

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FATE AND EFFECTS OF CONTAMINANTS IN MARINE ECOSYSTEMS AND ORGANISMS

Douglas A. Wolfe*

The general objectives of the National Oceanographic and Atmospheric Administration's (NOAA) energy resources project on fate and effects are to identify and conduct laboratory and field research studies that will facilitate the assessment of potential environmental impacts from petroleum releases in coastal marine ecosystems. This effort does not have a site-specific orientation; instead, it is focused on the fundamental processes that control the distribution, transport, and effects (including physiological and ecological aspects) of petroleum components, toxic metals, and other contaminants in coastal ecosystems. It is expected that the results of the process-oriented studies conducted under this project will have broad applicability to environmental problems in widely separated geographic areas.

This energy resources project represents only a small portion of NOAA's overall effort on fate and effects of contaminants in marine systems. Within its various research laboratories, the National Marine Fisheries Service (NMFS) conducts a wide variety of marine environmental research projects. These projects are directed toward defining the environmental requirements of fishery resource organisms and the effects of environmental additions or alterations to those resources. NOAA is also managing for the Bureau of Land Management the outer continental shelf environmental assessment program for environmental studies in support of petroleum exploration and development on the Alaskan Continental Shelf. In addition, two other energy resources projects are managed by NOAA: environmental assessments of the Northern Puget Sound and the Buccaneer oil field in the Gulf of Mexico. A high degree of interaction occurs throughout these various activities to minimize redundancy of effort and to promote effective research planning in all aspects of NOAA's program.

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The technical development plan (NOAA 1975) for the energy resources project on fate and effects identified four major tasks to be undertaken:

1. Establishment of an NOAA national analytical facility for analysis of petroleum hydrocarbons and toxic metals in the marine environment

2. Synthesis of existing information and identification of research needs on fate and effects of toxic metals and petroleum hydrocarbons in subarctic marine ecosystems

3. Laboratory research on specific effects or transport processes involving petroleum hydrocarbons and toxic metals in marine ecosystems

4. Experimental ecosystem research on ecological responses to additions of petroleum hydrocarbons.

During the first year, the efforts of this project on fate and effects were oriented mainly toward establishment of suitable in-house analytical support capability for NOAA's overall environmental program and the planning for future studies on fate and effects. In addition, laboratory studies were undertaken on the interactive effects of petroleum and polychlorinated biphenyl (PCB) exposure in fish and on the interactions of petroleum and metals in seawater-sediment systems.

NOAA NATIONAL ANALYTICAL FACILITY

The NOAA National Analytical Facility (NNAF) was established in August 1975 within the Northwest and Alaska Fisheries Center of NOAA's NMFS. Procurement of equipment and recruitment of staff began immediately. By March 1976, analytical work had been started by the full-time staff of four chemists under the direction of Dr. William MacLeod. At its Seattle location, NNAF has the added advantage of direct association with a multidisciplinary research staff engaged in experimentation on contaminant-induced changes in marine biota. This interaction will promote the realistic evaluation of analytical results in terms of observed effects on marine organisms.

The NNAF has six primary purposes:

1. Recommend the kinds of analyses needed to fulfill the objectives of environmental projects (e.g., in petroleum hydrocarbon analysis, should emphasis be placed on total hydrocarbons or on discrete fractions thereof?)

2. Undertake the examination and analysis of samples of biota, water, or sediments from both field and laboratory projects

3. Adapt and, where necessary, develop methods required by current needs of NOAA programs

4. Interpret data in relation to the biological and ecological importance to the biota and place them in the appropriate perspective for administrative decisions and actions

5. Adapt new methodology as objectives and needs of NOAA programs change

6. Provide contract liaison and quality control when analyses are conducted by other laboratories.

Major equipment acquisitions consist of a Finnegan 3200 gas chromatograph-mass spectrometer-data system (GC-MS), a scanning electron microscope, two automated gas chromatographs, a high-pressure liquid chromatograph, and a fluorescence spectrometer. These acquisitions supplement an extensive inventory of equipment already available within the Fisheries Center. With appropriate sample collection and preparation techniques, they will permit the reliable analysis of organic compounds at less than ppb levels.

Considerable effort was invested in training personnel on existing techniques and procedures, including revision and modifications where necessary. Much of the analytical work in the next few years will involve hydrocarbon residues in sediment and tissue samples. The NNAF staff has studied various analytical procedures for such samples, e.g., procedures of Warner (1976), Rohrbach and Reed (1975), and those under consideration by the Bureau of Land Management (1975). After careful consideration, the Warner procedures were provisionally adopted for training and testing. They have proven to be generally convenient and useful with certain notable exceptions. A major problem arose with the use of teflon-lined centrifuge tubes in the alkaline tissue digestion at 90°C. The linings failed to seal reliably, causing serious losses of moderately volatile hydrocarbons. This problem was solved by digesting overnight at lower temperatures. The silica gel chromatographic cleanup step also was troublesome. Mussel and snail tissue extracts effectively plugged the column. Alternative procedures are under investigation to alleviate this problem. Fortunately, our revision of the Warner method does not require separation of the aromatics into two fractions for GC analysis. Our GC column techniques permit the aromatics to be analyzed with good resolution in a single gas chromatogram. Recoveries of 85-90% for the examined hydrocarbons have been obtained from spiked control samples at a minimum average concentration of 5 mg of individual compound/g wet tissue. Presently, the GC sensitivity is 0.25 mg absolute amount of a single compound. Analytical methods are detailed in the report produced by NNAF under contract to NOAA's energy-related Puget Sound project (MacLeod *et al.*, 1976).

Studies undertaken by NNAF to date include the development and application of analytical techniques for hydrocarbons in biological samples of snails and mussels (MacLeod et al., 1976, 1977). In addition, NNAF performed the analyses for the experiments on hydrocarbon-PCB interactions in salmon, which were also supported under this energy resources project (Gruger et al., 1977a, 1977b).

FATE AND EFFECTS SYMPOSIUM

On November 10-12, 1976, NOAA and EPA cosponsored a major symposium, held in Seattle and entitled, "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms." The symposium, which was attended by approximately 350 persons from several countries, was intended to provide a vehicle for documenting the current status of research on the fates and effects of petroleum in the marine environment and for identifying areas most in need of future research. Twelve recognized experts were invited to present summary reviews on various aspects of petroleum in the marine environment (see Exhibit 1). In addition, to provide a detailed status of current research, contributed papers were solicited from the research community-at-large on this topic. Seventy-one abstracts were received in response to this solicitation, and 58 of these were selected by the symposium planning and editorial committee for actual presentation. Fifty-one manuscripts were submitted for publication in the symposium proceedings, and 35 of these were accepted by the committee. The final proceedings volume (Wolfe, 1977) includes the invited and the accepted contributed papers, as well as the panel discussion, which took place on the last day of the symposium. During this discussion, the panel and the audience expressed their thoughts on where the research priorities of the future ought to lie.

In the following paragraphs, I have attempted to tabulate the opinions expressed during the panel discussion on research needs. This list is clearly not an exhaustive itemization of all the imaginable research topics that will be of value to researchers and environmental decision makers over the next several years. Instead, it represents the current thinking of a few, highly perceptive scientists who have been heavily involved in research or research management in this topical area for several years. No attempt has been made to rank these suggested research areas and recommended approaches.

1. Acute toxicity bioassays should generally be de-emphasized in favor of longer term exposures and sublethal effects. Earlier static bioassays should be repeated using flow-through systems to improve estimates of species' relative sensitivities. Additional species or life stages suspected to be highly sensitive should be tested. Larval and juvenile stages still appear to be best candidates for research animals in effects studies. Petroleum effects on birds should be assessed under realistic

EXHIBIT 1: INVITED SPEAKERS AND TOPICS AT THE SYMPOSIUM ON FATE AND EFFECTS OF PETROLEUM HYDROCARBONS IN MARINE ECOSYSTEMS AND ORGANISMS

1. Oil Spills in the Alaskan Coastal Zone - The Statistical Picture. J.R. Harrald, D. Boyd, and C.C. Bates, U.S. Coast Guard, Washington, D.C.

2. Hydrocarbons in the Water Column. D.G. Shaw, University of Alaska Institute of Marine Sciences, Fairbanks, Alaska.

3. Oil Slick Movement in Alaskan Waters. P.J. Kinney, Kinnetic Laboratories, Santa Cruz, California.

4. Oil Slick Weathering. C.D. McAuliffe, Chevron Oil Field Research Co., La Habra, California.

5. Biodegradation of Aromatic Petroleum Hydrocarbons. D.T. Gibson, University of Texas, Austin, Texas.

6. Bioconversions and Metabolism of Petroleum Hydrocarbons. D.C. Malins, NMFS Northwest and Alaska Fisheries Center, Seattle, Washington.

7. Accumulation and Turnover of Hydrocarbons in Marine Organisms. R.F. Lee, Skidaway Institute of Oceanography, Savannah, Georgia.

8. Food Web Transport of Petroleum Hydrocarbons. J. Teal, Woods Hole Oceanographic Institute, Woods Hole, Massachusetts.

9. Comparative Toxicities of Petroleum Hydrocarbons in Marine Organisms. S.D. Rice, NMFS Auke Bay Fisheries Laboratory, Auke Bay, Alaska.

10. Sublethal Effects of Petroleum Hydrocarbons, J. Anderson, Battelle Marine Research Laboratory, Sequim, Washington.

11. Morphological Abnormalities Produced by Hydrocarbon Exposure. J. Hawkes, NMFS Northwest and Alaska Fisheries Center, Seattle, Washington.

12. Ecological Effects of Petroleum in Marine Systems. A.P. Michael, University of Massachusetts Marine Laboratory, Gloucester, Massachusetts.

exposure conditions (both long- and short-term). All bioassay experiments, whether acute or chronic, should use realistic dose-time exposure regimes (based on concentrations and compositions likely to occur in natural systems) to assess potential effects. Uptake and turnover of compounds should be documented by thorough chemical analysis of tissues, and subsequent experimental and analytical emphasis should be on those specific compounds actually accumulated by the exposed organisms (instead of on some measure of total hydrocarbons).

2. Long-term effects to be studied should emphasize studies on:

   - Behavior (feeding, reproductive, migrational)
   - Carcinogenicity
   - Cellular ultrastructure
   - Chemosensory mechanisms
   - Disease incidence and susceptibility
   - Enzyme activities
   - Growth
   - Immune responses
   - Mutagenicity
   - Reproduction.

3. Effects of petroleum components, other than hydrocarbons and intermediary metabolites of hydrocarbons, should be examined. The relative toxic effects of various petroleum constituents should be assessed. Past emphasis has been on hydrocarbons per se mainly because of the relative ease of analysis, not because they are the known direct causative agents in manifesting the effects.

4. Potential transfer through food webs should be examined for hydrocarbon metabolites and nonhydrocarbon components of petroleum. Persistence of metabolites in organisms, which could be examined through the use of radiotracers, is a corollary problem.

5. Various physical and chemical forms of contaminants, including chemically complexed forms, should be tested for relative bioavailability, metabolism, and toxicity to marine organisms. The natural occurrence and rates of formation of such complexes should be determined in marine systems.

6. Petroleum-sediment interactions should be characterized, and the transfer of sediment-associated petroleum constituents to organisms should be quantitated.

7. Synergisms between different types of contaminants and between contaminants and physical parameters (e.g., temperature and salinity) should be examined.

8. Field studies should be designed and conducted to corroborate laboratory results. Such studies might involve intentional perturbation under controlled conditions, long-term studies of oil spills, and ecological and physiological studies of chronically polluted areas and areas of natural seeps. Field experiments require very careful and thorough planning to ensure that causality of effects will be documented and that sampling frequency and intensity are adequate to demonstrate statistically a desired level of effect.

9. Models of contaminant cycling in the environment should be refined to include the kinetics of physical and biological transport processes (e.g., solution, evaporation, adsorption, bioaccumulation) and chemical and biological modification processes (e.g., photooxidation, biodegradation). Compositional changes in petroleum should be determined and should serve as a basis for experimental design in effects studies.

The foregoing suggestions and recommendations will be useful in planning and evaluating future research. However, detailed proposals must be evaluated in the context of work already underway at the time, and in terms of the availability, competency, and operational readiness of investigators to perform the work.

INTERACTIONS BETWEEN PETROLEUM AND POLYCHLORINATED BIPHENYLS IN SALMON

In marine environments, numerous types of contaminants exist together, including petroleum hydrocarbons and PCBs. These xenobiotics are expected to produce a variety of biological alterations in marine organisms, such as biochemical and morphological changes in cellular and subcellular systems, that could possibly influence their survival rate. Studies of the effects of contaminant mixtures are important for judging possible harm that may come to marine resources exposed simultaneously to such compounds.

A study was designed to delineate nonlethal effects of chlorobiphenyls and petroleum hydrocarbons on salmon (Oncorhynchus species) when mixtures of each xenobiotic were included in the diet separately and together (Gruger et al., 1977a). Specifically, the research included studies of accumulations and depurations of the test compounds, the influences on an enzyme system (aryl hydrocarbon hydroxylases) associated with their metabolism, and associated changes in cellular structures of key organs.

Coho salmon (Oncorhynthus kisutch) and chinook salmon (O. tshawytscha) were exposed to two mixtures of pure chlorobiphenyls and petroleum hydrocarbons, separately and together, at 1 ppm and 5 ppm of each mixture in Oregon moist pellets. Chemical analyses of coho salmon tissues indicated that the chlorobiphenyls accumulated in the liver, kidney, and muscle tissues; hydrocarbons were not detected in these areas.

Microscopic examinations indicated that the chlorobiphenyls and hydrocarbons had no effect on the morphology of gill tissue; however, there was a possible dysplasia of the surface cells of skin. The effects of chlorobiphenyls on liver were variable; in one chinook salmon there was severe vesiculation of hepatocytes, as well as whorled intracellular membranes similar to neoplastic cells in rats. Chinook salmon treated with both chlorobiphenyls and chlorobiphenyls and hydrocarbons together exhibited mucosal sloughing in the intestine. No tissue damage was observed by light microscopy in fish receiving hydrocarbons alone. It is provisionally concluded that chlorobiphenyls and hydrocarbons have a synergistic effect on the severity of sloughing of the intestinal epithelium and exudation of mucus.

Aryl hydrocarbon [benzo (a) pyrene] hydroxylase activities of liver microsomes were compared among test and control salmon of both species in order to determine whether alterations in enzyme activities occurred in relation to the exposures (Gruger et al., 1977a; 1977b). Optimal reaction conditions were established for measurements of aryl hydrocarbon hydroxylase activities in salmon hepatic microsomes; the conditions indicated differences compared to mammalian microsomes and differences in pH for the reactions for coho salmon compared to chinook salmon.

Induction of the aryl hydroxylase enzyme occurred in coho salmon within 2 weeks of exposure to the hydrocarbons. In addition, the induction was potentiated by the presence of the chlorobiphenyls, which by themselves had no effect on the enzyme activity. A synergistic effect was indicated for the test compounds on the induction of aryl hydrocarbon hydroxylase in coho salmon livers. In chinook salmon, the activity of aryl hydrocarbon hydroxylase in hepatic microsomes was depressed by the chlorobiphenyls and hydrocarbons administered, both separately and together. The results provide evidence indicating that different salmonid species respond differently to chlorobiphenyls and petroleum hydrocarbons in food in terms of the hepatic aryl hydrocarbon hydroxylase system.

INTERACTIONS BETWEEN PETROLEUM AND TOXIC METALS IN SEDIMENT-SEAWATER SYSTEMS

Sediments constitute a very large reservoir for metallic elements in aquatic environments, and the steady-state partition coefficients and exchange rates depend upon redox potential, pH, and other chemical characteristics of the sediments, interstitial water, and overlying water column. Contamination of the benthic or intertidal marine system by petroleum could change this chemical microenvironment, thereby affecting the distribution and biological availability of metals to demersal and benthic organisms.

To measure the magnitude of this potential secondary impact of petroleum, various concentrations of crude oil will be added to experimental mixtures of marine sediments and sea water that have equilibrated with respect to distribution of selected metallic elements. After oil impaction, the rate of change of elemental concentrations in the water and the ultimate distribution of the metals between sediment and water will be measured. Thus, the effects of known compositions and concentrations of oil on the exchange rates and partition coefficients of metals between water and sediments will be determined. The results of this study will be correlated with existing information on bioaccumulation of metallic elements to assess the significance of petroleum contamination with regard to accumulation and toxicity of metals in the marine environment. This experiment is currently in the initial phase of study, and results are not yet available.

DESIGN OF EXPERIMENTAL ECOSYSTEM STUDIES

Carefully controlled studies of petroleum behavior and impacts under large-scale experimental conditions in relatively undisturbed ecosystems provide the most feasible means of assessing potential petroleum impacts at the population or ecosystem level of organization and for testing and verifying the results of laboratory experiments under field conditions. Experimental releases of oil in an isolated portion of a larger study area must, however, be carefully designed to approximate closely the impact of oil on the whole natural ecosystem. In addition, the experimental system must be well understood ecologically prior to initiation of experimental releases of petroleum. It should be thoroughly characterized in terms of all those parameters that might significantly affect interpretation of data on a specific hypothesized impact.

Such a characterization probably should produce reasonable understanding of species productivity, variability in community structure and diversity, trophic relationships, and seasonal successionary changes. Processes affecting the transport and distribution of petroleum should also be characterized, including water circulation, sedimentation rates and distributions, and air-sea interactions such as evaporation and aerosol formation. Experiments should be designed to document and measure both short-term effects and the effects of persistent low-level petroleum exposure in nearshore ecosystems, ranging from the subtidal littoral zone through the intertidal zone to the adjacent beaches or wetlands. These studies should include shore birds and mammals and other terrestrial trophic elements that may become exposed to the petroleum.

The inherent complexity and natural variability of natural ecosystems require that studies of this type be designed with a great deal of care to ensure that:

- Sampling frequency and intensity are adequate to document hypothesized effects to within specified statistical limits

- The parameters selected for measurement are appropriate to demonstrate causality for a particular hypothesized effect.

A major constraint limiting the ability to achieve adequate design of ecological perturbation studies is our lack of understanding about how the ecosystem functions naturally. That is, what are the important causative interactions and how do they interrelate quantitatively?

Given this lack of understanding, it becomes essential to limit the scope of experiments to those specific hypothesized impacts that we can realistically expect to test with a rigorous sampling design. The use of experimental perturbation studies under natural field conditions should be restricted to those issues that cannot be addressed adequately under accidental oil spill conditions. Thus, the gross effects of smothering on an oiled intertidal zone or the recolonization of a denuded shore by mussels are not questions warranting the expense of designing and conducting controlled field experiments on oil impacts. These dramatic effects can be readily quantified at the scenes of accidental oil spills (although sampling of such phenomena has rarely, if ever, been designed to provide additional insight on the sequence of causal mechanisms involved in the process). On the other hand, controlled spills, coupled with carefully conceived manipulations of the ecosystem, may be the only means of verifying the relative importance of specific food-chain relationships in transferring contaminants between organisms under actual field conditions, or for separating the effects of predation and pollutant toxicity on population stability of a given species. The controlled experimental spill provides realism in the introduction, the natural transport pathways, the chemical compositions, etc., of petroleum in a marine system. Concurrently, it provides a stage for conducting ecological manipulations that can document mechanistic causality for selected processes of interest.

Numerous conferences on biological effects have decried the validity or applicability of laboratory results to field situations and have almost casually recommended controlled field experiments as the logical approach to achieve the desired realism.* A workshop sponsored by Exxon (1974) also espoused the controlled field experiment as a means of studying chronic effects of oil in marine systems. It is clear from the foregoing discussion that the realities of ecosystem complexity and natural variability severely restrict the real utility of controlled field experiments and demand a high degree of planning and experimental design prior to implementation.

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* See, for example, recommendation no. 8 above, which was put forth on several occasions at the recent fate and effects symposium sponsored under this project (Wolfe, 1977).

As a first step toward the necessary planning, NOAA (through a contractor) is conducting design feasibility analyses for various experimental approaches and ecological hypotheses that might be appropriately tested in field perturbation experiments with petroleum. Results of the study should be available this year.

Future work under this project will continue to be directed toward understanding specific processes and mechanisms affecting the movement, distribution, and effects of contaminants in marine systems. It is expected that, when adequate design criteria are developed, controlled field experiments will be utilized interactively with laboratory experiments for this purpose.

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DISCUSSION

From the floor: Could you give us a rationale for your choice of hydrocarbon levels and PCB levels in your salmon experiment?

Dr. Wolfe: We may not have conducted adequate studies on the threshold levels of PCB and petroleum required to induce the enzymatic activities. The selected concentrations are representative of total hydrocarbon levels found in marine organisms under natural environmental conditions. The PCB levels are still high. Again, the purpose of the experiment was to determine whether there is a synergistic interaction between the two components, so we wanted to emphasize the effects of the PCBs.

Dr. Lefcort: Would you elaborate on your plans for these field studies?

Dr. Wolfe: In the beginning, we thought that we could characterize a natural marine system to an extent that we could then hypothesize certain effects of petroleum and conduct a deliberate spill to verify or refute those hypotheses. Consequently, the project was oriented towards deliberate spills, and the ongoing feasibility analyses are still directed toward deliberate spills.

NOAA is currently undertaking a spills of opportunity program funded under the outer continental shelf environmental assessment program. Under this program, NOAA has attempted to identify specific research questions that might be feasible under opportunistic conditions. We have assembled four spilled-oil research teams, which are able to respond on the basis of a midnight phone call to a spill that occurs almost anywhere at anytime.

So far, the objectives that have been put forth for opportunistic spills are confined to questions of physical transport and physical behavior of oil in a marine environment. At this time, we have not designed any biological experiments that we considered feasible.

Dr. Barrientos: Sometime last November, Craig Hooper put together the environmental impact statement to do a control on this experiment. Are these field experiments related to that at all?

Dr. Wolfe: A deliberate spill was designed under the outer continental shelf program. This program reached the point of a license request draft to be presented to EPA. The proposal was to assess the physical parameters of petroleum behavior in the marine environment in the Gulf of Alaska. That draft was undergoing review when the Argo Merchant went aground. Because of several different constraints resulting from that disaster, that proposal has been postponed.

LABORATORY AND FIELD STUDIES ON THE LONG-TERM EFFECTS OF PETROLEUM HYDROCARBONS ON BENTHIC MARINE INVERTEBRATES

J.W. Anderson, R. Riley, R.M. Bean, J.W. Blaylock, and S.L. Kiesser*

As a result of oil production from the Alaskan outer continental shelf, the Pacific Northwest is one of the areas of the United States most likely to receive increased inputs of petroleum hydrocarbons (PHC) from transport, port operations, and refining. There is the possibility that exploration on the coasts of Oregon and Washington will indicate the potential for drilling; thus, production-related inputs are possible. Present and future activities associated with this energy source necessitate research on the potential impacts of oil in the Pacific Northwest marine environment. Although the results of recent studies on the fate and effects of PHC in the marine environment have strengthened our understanding of some aspects of oil pollution, we are not yet to the point at which an accurate prediction of the effects of acute or chronic oil inputs can be made.

To define more clearly the potential for petroleum to exert significant long-term environmental effects in marine sediments, studies on the fate of crude oil in sediments are being conducted. These interdisciplinary studies involve aspects of physical organic chemistry, marine biology, and microbiology. Their principal objectives are to determine the rates and mechanisms of degradation of petroleum in the marine environment, to determine which component types are persistent, and to correlate such information, where possible, with observed effects on the marine biota.

To meet the analytical objectives, considerable effort has been directed toward development of analytical methods for the separation, identification, and quantitation of classes of compounds and individual compounds that comprise saturate and aromatic fractions of Prudhoe Bay crude oil. Analytical methodologies developed on Prudhoe Bay crude oil have been appropriately modified for analysis of petroleum compounds in sediments, tissues, and laboratory exposure systems.

In this paper, we report on those methods that are being used to meet some of the above needs. These techniques are being used to

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separate, identify, and quantitate the components of Prudhoe Bay crude oil and to quantitate these components in sediment samples contaminated with the oil as a part of laboratory and field depuration studies. The methods used in these studies employ the techniques of liquid-column chromatography, capillary-column chromatography, gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and infrared spectroscopy (IR).

To provide the scientific data base necessary to evaluate the impact of oil in the marine environment, large-scale programs incorporating a "team effort" are required. Marine biologists, with expertise in different fields (e.g., ecology, physiology, biochemistry), must work closely with analytical chemists, and each should utilize the best available techniques while attempting to develop even better approaches. From a review of the literature on fate and effects of oil, much of which was updated at a recent symposium in Seattle (*Fate and Effects of Petroleum in Marine Ecosystems and Organisms*, ed. D. Wolfe, NOAA), it appears to us that at least four major questions remain to be answered:

1. When exposed to oil, which specific PHCs are retained longest and to the greatest extent in organisms and sediment?

2. Are sediment-sorbed PHCs available to organisms feeding and/or burrowing in the substrate, and is there biomagnification?

3. As a result of oil toxicity or chemical interference, are larval benthic organisms eliminated from contaminated sediments (lack of recruitment), and what are the time and concentration factors involved?

4. What is the threshold PHC level at which long-term exposure no longer alters marine communities nor reduces the capability of sensitive organisms to grow and reproduce normally?

We believe that the analytical developments and biological approaches discussed in this paper provide the scientific data base necessary to answer all or part of these questions.

MATERIALS AND METHODS

We have categorized this section according to those analyses that involve analytical chemistry and those that are biologically oriented.

Analytical Chemistry

In this section, we describe the analysis of PHCs in the flowing sea water system, the analysis of Prudhoe Bay crude oil, field and laboratory studies of depuration of oil in sediment, analysis of total oil in sediments, and field and laboratory studies of separation and quantitation of compounds in sediments.

Analysis of PHCs. Investigations of the toxicity of crude and refined oils in sea water have been underway at Battelle-Northwest's Marine Research Laboratory for several years. A principal means of carrying out these studies has been the use of continuous flow oil exposure apparatus developed at the Battelle-Northwest Marine Research Laboratory (Vanderhorst *et al*., 1976a). To determine total oil concentrations and to quantitate individual hydrocarbons at the concentrations currently being used, two separate analytical techniques have been developed and applied. The first technique involves extraction of petroleum-contaminated sea water samples with helium, and injection of the helium extract into a gas chromatograph for determination of volatile hydrocarbon component concentrations. This method is about three orders of magnitude more sensitive than conventional methods. Bean and Blaylock (1977) recently described it in detail, and Vanderhost *et al.* (1976a) applied it in a study of No. 2 fuel oil toxicity utilizing oil concentrations of less than 200 ppb.

The second analytical method allows the characterization of low concentrations of oil in sea water and involves the use of resin adsorption. Approximately 10 ℓ of sea water from the flow-through oil exposure apparatus are pumped through 3/8 in. columns containing XAD-2 macroreticular resin. The resin has been shown to efficiently adsorb the oil components from the sea water even at low concentrations. Recovery of oil from a sea water suspension containing 115 ppb Prudhoe Bay crude oil was 92.4 ± 1.7% efficient, based on triplicate determinations. Analysis of the adsorbed oil is accomplished by eluting the column with carbon tetrachloride and determining the IR absorbence of the eluate. It has been found that the analytical columns can be regenerated at least nine times (by washing with methanol, then water) before new resin must be employed.

Analysis of Prudhoe Bay crude oil. The separation of Prudhoe Bay crude oil into saturate and aromatic compound fractions was adapted from a silica gel chromatography technique described by Warner (1976) and modified by MacLeod *et al*. (1976). Two fractions (saturate and aromatic) were collected. Each fraction with its appropriate internal standard was concentrated and analyzed by gas chromatography on a 30 m SE-30 glass capillary column (J & W Scientific) programmed from an initial temperature of 70° to 250° at a rate of 4°/min. Compounds in the saturate and aromatic fractions were identified by peak enhancement analysis with known standards. A Hewlett Packard 5980A quadrapole mass spectrometer operating in the argon chemical ionization mode and employing single ion monitoring was used to determine approximate retention time ranges for specific compound types within the aromatic fraction.

Field and laboratory studies of depuration of oil in sediment. A surface layer of oil (except controls) was deposited in trays containing coarse beach sediment at a level of 4% of the sediment weight. Forty-eight hr of tidal flushing (four cycles) were used to remove excess oil from the surface of the sediments. After the 48 hr flushing period, the trays were placed in the field. Since the initiation of the experiment on April 17, 1976, samples were collected and frozen approximately every 2 months. Samples were treated and analyzed as described below.

Depuration of oil in sediments (laboratory study). The laboratory study was initiated early in 1976 by introducing the fine sediment mixed with 1% fresh Prudhoe Bay crude into an aquarium. An additional aquarium, serving as a control, was charged with fine-textured sediment free of Prudhoe Bay crude. The depth of sediment was approximately 4 in., and fresh sea water was allowed to continuously flow over the sediments in both aquaria. Triplicate grab samples were removed and frozen at intervals spanning 180 days. Hexane and CCl_4 extracts of the sediments were analyzed by IR and HPLC.

Analysis of total oil in sediments by IR. Concentrations of total oil in sediments were determined on carbon tetrachloride extracts of sediments with a Beckman Acculab 6 spectrophotometer by comparison of the amount of absorbance obtained at 2930 cm^{-1} to a calibration curve (API, 1958).

Separation and quantitation of compounds in contaminated sediment (field study). Triplicate 20 g samples of sediment were extracted for 24 hr in glass bottles containing teflon-lined caps, 20 g of anhydrous sodium sulfate, and 50 mℓ of hexane (Burdick & Jackson). Appropriate amounts of each sample were concentrated to 1 mℓ and column-chromatographed according to the method of Warner (1976). Saturate and aromatic fractions were analyzed by capillary column chromatography as previously described, employing 2,6,10-trimethyldodecane and hexamethylbenzene as internal standards. Peak areas and internal standard calibrations were determined with an Autolab Systems IV Computer integrator.

Total oil and separation and quantitation of classes of compounds from oil-contaminated sediment (laboratory study). Three μℓ of hexane extracts of sediment obtained as previously described were analyzed by HPLC, utilizing three series-coupled 1/8 in. x 2 ft Durapak oxypropionitrile on porasil C columns (Waters Associates), using hexane as mobile phase and 2.0 mℓ/min flow rate. The columns were previously validated with the model compounds dodecane, benzene, naphthalene, and anthracene to demonstrate separation of hydrocarbons into saturate, monoaromatic, diaromatic, and polyaromatic fractions. The quantitative analysis of the aromatics in sediment samples was performed with a calibration curve from a Prudhoe Bay crude aromatics fraction prepared according to an ASTM method (Vanderhorst et al., 1976a). Saturate fractions were detected with a Waters R-401 differential refractometer, and the aromatic fractions were detected with a Schoeffel variable wavelength ultraviolet (UV) detector set at 221 μm.

Biological Analysis

In this section, we describe our flowing sea water and flowing exposure systems.

Flowing exposure system. Our flowing sea water system for exposing organisms to low levels of soluble plus finely dispersed PHC has been previously described by Vanderhorst *et al*. (1976a). Prudhoe Bay crude oil was used as the source of contamination, since it is likely to be one of the primary products delivered to this area, and other researchers in Alaska and the Pacific Northwest are utilizing the same oil in their research. The hydrocarbon delivery system was adjusted to deliver 0.22-0.24 ppm total hydrocarbons, determined by IR, as described above.

A mixture of intertidal sediments (3 cm and less in size) was collected at mean low tide for transfer into three fiberglass exposure tanks (90 x 40 x 30 cm). One of the tanks received clean flowing sea water, while the other two received a mixture of sea water and an oil-in-water dispersion prepared in the continuous-flow apparatus (rate of 1.5 ℓ/min).

Anonyx nugax (Amphipoda: Crustacea) cultures of 100 individuals were introduced into the control tank and one of the exposure tanks on October 4, 1976. Uptake of total naphthalenes and long-term effects of low-level contamination on growth and reproduction were monitored. A 1 mm screen was fastened over the standpipe of the control tank to prevent the amphipods from escaping. Outfall baskets were constructed on exposure tanks to eliminate the buildup of a surface oil sheen, while still retaining the animals. A second exposure tank was stocked with 100 amphipods 4 weeks later as a replicate. Uptake of total naphthalenes in the tissue of exposed amphipods was measured after 7, 10, and 14 weeks by the UV spectrophotometric technique described by Neff and Anderson (1975). Effects on growth were determined by total length and wet weight measurements.

Sediment exposure systems. Using a system previously described by Anderson *et al*. (1977), peanut worms (phylum Sipunculida) were exposed to oil-contaminated sediment in the laboratory. Sediment boxes, made of PVC or fiberglass with bottoms covered by fiberglass mesh, were used to hold sediment that had either been oiled on the surface or mixed thoroughly with oil. These boxes and the burrowing worms (*Phascolosoma agassizii*) were held up to 2 weeks in flowing clean sea water and simulated tidal fluxes periodically drained the substrate via the bottom mesh. Analyses were conducted during the experiments to determine naphthalenes in the sediment and tissues and total hydrocarbons in the sediment.

A similar approach has been utilized in field experiments, involving oiled sediment and three species of benthic organisms. Sipunculids and two clam species (*Macoma inquinata* and *Protothaca staminea*) were placed in boxes containing either clean or oiled sediment (622 ppm total hydrocarbons by IR) and these were placed in the intertidal zone for 40 days. After 40 days, tissue samples of each species were analyzed by GC, following the methods of Warner (1976).

Beginning in March 1976, sediment boxes containing clean and oil-contaminated substrate were placed in the intertidal zone on the sand spit east of the Sequim laboratory. The first two installations (March and April, 1976) consisted of six boxes each, four of which were contaminated by Prudhoe Bay crude oil via surface coating. Using a tidal flux system in the laboratory, a layer of oil (4% vertical velocity sediment) was allowed to impact the surface for about 1 hr, before a series of high (above surface) and low (below the box) tides were initiated. After 48 hr (four tidal flushes) in the laboratory during which excess oil was floated off the sediment, the boxes were transferred to the field site and installed flush with the natural sediment. A third installation in November 1976 was treated in a similar fashion, but sediment was mixed thoroughly in a fiberglass-coated cement mixer instead of receiving oil on the surface only.

The first installation has now been in place for nearly 1 year, during which numerous samples have been taken for analyses of specific hydrocarbon composition and presence of recruited biota. Methods of chemical analyses are discussed above and the results of initial chemical characterization by capillary gas chromatography (Cap-GC) and GC-MS are presented below. Analyses of benthic organisms found in the sediment samples are still underway, but the numbers of two prominent bivalves present in control and exposed sand in June and August 1976 will be discussed. *Cooperella subdiaphana* and *Mysella tumida* were selected in this preliminary report, since they clearly represent recruited organisms and not just transient inhabitants of the sediment. In the future, we will be able to report the number of species and individuals in each box at several time intervals over a period of years. It is important to note the significance of being able to correlate detailed and quantitative hydrocarbon analyses with the abundance and diversity of benthic organisms over an extended period of time.

RESULTS

We describe below our results for the above methods.

Analytical Chemistry Results

The results of our analyses of the flowing exposure system, Prudhoe Bay crude system, depuration of oil in sediments, are summarized in the following paragraphs.

Flowing exposure system. Using the He-GC technique for determination of monocyclic aromatics, the flowing oil delivery system has been characterized as shown in Exhibit 1. Analyses on December 17, 20, and 21 show the remarkable consistency of component concentrations in our exposure system. As reported by Rice *et al*. (1977), monoaromatics tend to remain in cold sea water to a greater extent than has been observed in laboratory studies or field investigations in temperate waters. The low molecular weight compounds represent a significant proportion of the hydrocarbons to which organisms are exposed in the system, and it is likely that this would be true for chronic and acute inputs to the marine environment of the Pacific Northwest. It should be noted that the total hydrocarbon (IR) values listed at the bottom of Exhibit 1 do not necessarily relate to totals of low molecular weight compounds. In fact, only a small percentage of the latter hydrocarbons are detected by IR, so these should be considered independent analyses. A realistic grand total would be somewhat less than the sum of these two separate analyses.

Resin column extractions of 10 ℓ samples from exposure water were conducted from September 14, 1976, to January 14, 1977, and are still in progress. Concentrations of total hydrocarbons were determined for the same three exposure tanks during this period. Analyses shown in Exhibit 2 have been sorted into three time intervals, as flow rates were altered to suit our experimental requirements. Tanks 4 and 5 were calibrated to deliver the same concentrations, except for the period of October 20, 1976, to November 1, 1976. Although only a slight decrease in concentration of Tank 4 was produced after October 19, 1976, a major decrease in Tank 6 was intentionally produced on October 20, 1976. The consistency within a given time period in a specific tank was very high, as shown by the standard deviations. One-way analyses of variations (ANOVA) among tanks at each time interval demonstrated that all concentrations were equal during the first 35 days, but later adjustments produced significantly higher concentrations in Tanks 5 (12-day period) and 6 (last 82 days). Without considering the adjustments, the means of Tanks 4 and 5 were relatively close over the entire 117-day period. It is important to remember the consistency of the system and the levels present in Tanks 4 and 5, since these contained the amphipod cultures to be discussed below.

Analysis of Prudhoe Bay crude oil. Chromatographic separation of Prudhoe Bay crude oil produced a saturate and an aromatic fraction. Compounds that were identified in the saturate fraction by gas-capillary column chromatography included the straight-chain hydrocarbons from C_{11} to C_{26}, pristane, and phytane. Pristane and phytane were baseline resolved from heptadecane and octadecane (see Exhibit 3). Identification and quantitation of compounds in the aromatic fraction have proved more difficult because of the lack in the availability of commercial standards and because many are present in Prudhoe Bay crude oil at much lower concentrations relative to the saturate compounds. Identifications that have been made are listed in Exhibit 4. Many compounds in the aromatic fraction were categorized by GC-MS according to compound types, i.e., monomethyl-, dimethyl-, and trimethylnaphthalenes, monomethyl-, C_2-, and C_3-phenanthrenes, etc. Thus, the GC-MS information has enabled us to estimate the concentration of hydrocarbon types.

EXHIBIT 1: ANALYSIS OF LOW MOLECULAR WEIGHT HYDROCARBONS IN THE FLOWING SEA WATER SYSTEM RECEIVING PRUDHOE BAY CRUDE OIL [All values are expressed as µg/l (ppb) and water temperature was 8° ± 0.2°C.]

Petroleum Hydrocarbons	AM 12/17/76 Tank 4	AM 12/17/76 Tank 5	AM* 12/17/76 Tank 6	PM 12/17/76 Tank 5	AM 12/20/76 Tank 5	AM* 12/20/76 Tank 6	AM 12/21/76 Tank 5	AM* 12/21/76 Tank 6
Benzene	25.1	24.8	64.4	40.1	45.5	77.0	62.1	82.9
Toluene	53.6	50.7	86.7	55.3	66.1	102.2	71.1	116.4
Ethyl Benzene	5.2	4.3	9.6	5.1	6.2	10.8	6.2	12.1
m and p Xylene	21.2	19.2	36.7	22.1	29.4	36.1	28.8	49.0
o Xylene	8.5	7.6	15.1	8.7	16.4	17.9	6.7	27.6
Trimethyl Benzenes	9.5	8.8	26.7	10.9	13.7	21.6	23.0	37.7
Saturates $<nC_{12}$	20.9	19.3	33.9	13.4	21.6	31.8	18.8	33.5
Total Analyzed	144.0	134.7	273.1	155.6	198.9	297.4	216.7	359.2
IR analyses (total hydrocarbons) of resin column extractions					130.0	310.0	130.0	260.0

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* Tank 6 was calibrated to produce a higher concentration during this period.

EXHIBIT 2: TOTAL HYDROCARBONS (ppm) IN THE FLOWING SEA WATER SYSTEM AS DETERMINED BY IR ANALYSES OF COMPOUNDS SORBED TO RESIN COLUMNS RECEIVING 10 ℓ OF WATER

		TANK #4			TANK #5			TANK #6		
Sample Interval	No. Days	No. Samples	M	S.D.	No. Samples	M	S.D.	No. Samples	M	S.D.
9/14/76-10/19/76	35*	17	0.31	0.141	17	0.22	0.084	17	0.24	0.10
10/20/76-11/1/76	12†	6	0.23	0.032	6	0.37	0.045	5	0.40	0.077
11/5/76-1/14/77	70†	16	0.16	0.055	16	0.16	0.045	16	0.32	0.126
9/14/76-1/14/77	117	39	0.24	0.126	39	0.22	0.032	38	0.30	0.122

* Concentrations in the three tanks not significantly different ($p<0.05$, one-way ANOVA).

† Concentrations in the three tanks different ($p<0.001$, one-way ANOVA).

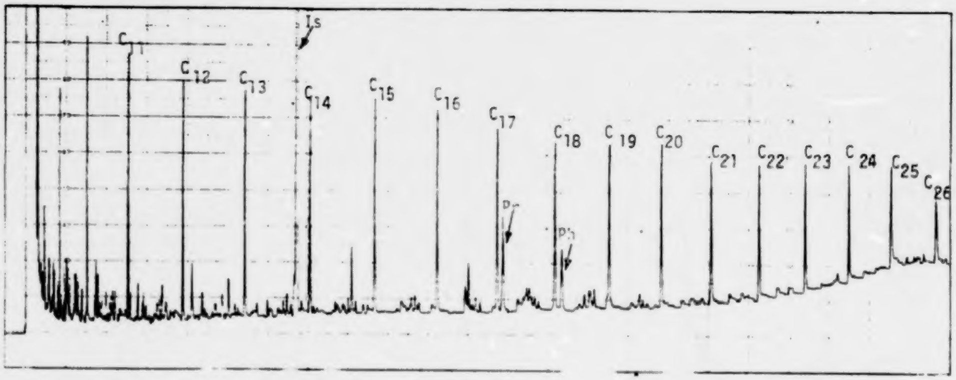


EXHIBIT 3: GAS CAPILLARY CHROMATOGRAM OF SATURATE FRACTION ($C_{11} \longrightarrow C_{28}$, saturate straight chain hydrocarbons, Pr = pristane, Ph = phytane, Is = 2,6,10-trimethyldodecane internal standard)

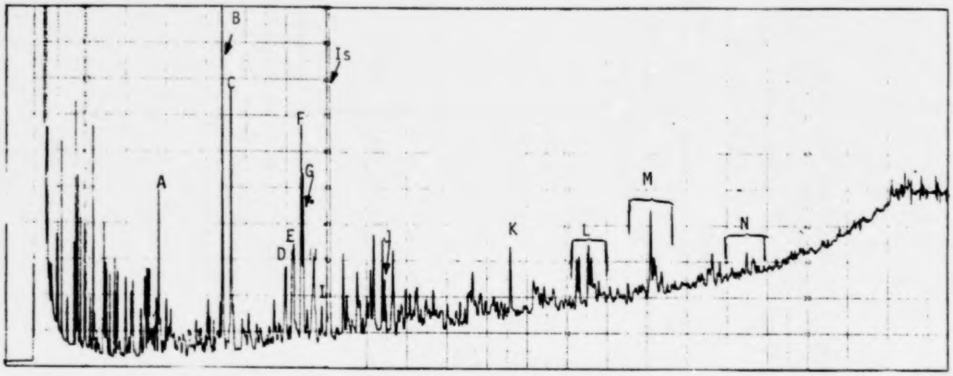


EXHIBIT 4: GAS CAPILLARY CHROMATOGRAM OF AROMATIC FRACTION

Key:

A. Naphthalene
B. 2-Methylnaphthalene
C. 1-Methylnaphthalene
D. 1-Ethyl + 2-Ethylnaphthalene
E. 2,6 + 2,7*-Dimethylnaphthalene
F. 1,3 + 1,6-Dimethylnaphthalene
G. 1,7*-Dimethylnaphthalene
H. 1,4 + 2,3 + 1,5-Dimethylnaphthalene
I. 1,2-Dimethylnaphthalene
J. 2,3,6-Trimethylnaphthalene
K. Phenanthrene
L. Methylphenanthrenes
M. C -Phenanthrenes
N. C -Phenanthrenes
Is. = Hexamethylbenzene internal standard

* 2,7 and 1,7 are suggested structures based on published literature on the law resolution gas chromatographic separation of dimethylnaphthalenes.

Depuration of oil in sediments. The above technique was applied to the field study of the depuration of the components of Prudhoe Bay crude oil from a coarse beach sediment. The concentrations of compounds in the saturate and aromatic fractions from sediment taken from the day of installation are listed in Exhibit 5. Dodecane, naphthalene, and phenanthrene were selected as representative saturate and aromatic components of oil, and their rate of depuration in the sediment was determined over a period of 7 months. The results are shown in Exhibit 6. Only 2.1%, 1.0%, and 2.6% of the original dodecane, naphthalene, and phenanthrene, respectively, remained in the surface portion of the sediment after 7 months of exposure of the sediment in the beach environment. These results were in excellent agreement with values of total oil obtained on the same samples.

The retention time of Prudhoe Bay crude as a function of total oil and compound class was determined on a fine-textured sediment under anaerobic conditions. We used the method employing IR and HPLC. The results of extraction of the sediment with carbon tetrachloride followed by IR analysis are shown in Exhibit 7. These data were based on analyses of three subsamplings from each of two sample replicates for each time period. The variance experienced among subsamples of the same jar (coeff.-3.7%) was less than the total variance (coeff. -5.8%). These data obtained for oil concentration indicate that the rate of depuration of oil from fine, undisturbed, anaerobic sediments can be very slow, on the order of several years.

Samples of hexane extract of the same sediment contaminated with oil when analyzed by HPLC (analysis time ∿20 min) produced a saturate fraction and a monoaromatic/diaromatic fraction, which was approximately 80% resolved. The data for the aromatics analysis are also shown in Exhibit 7. The plot suggests slow depuration of these components, but at a rate somewhat faster than the total oil. Correlation of the data with the linear regression (dashed line, Exhibit 3) was better ($r^2 = 0.44$ vs 0.31) than for the total oil curve.

Results of Biological Analysis

In the following paragraphs, we summarize the results of our analyses of the flowing exposure system and field exposure of oiled sediments.

Flowing exposure system. As shown in Exhibit 8, the amphipod Anonyx nugax accumulated a significant amount of naphthalenes during constant exposure to the Prudhoe Bay crude oil extract. Considering the individual variations, exhibited by the standard deviations, the animals reached a threshold of accumulation by 7 weeks of exposure. There was no significant increase over this level of contamination at the 10- or 14-week interval. From additional unpublished data (Anderson and Kiesser), there is a strong trend for the levels of specific compounds in the tissues to change from approximately equal distribution of naphthalene (N), methylnaphthalenes (MN), and dimethylnaphthalenes

EXHIBIT 5: CONCENTRATIONS OF SATURATE AND AROMATIC HYDROCARBONS (ppm) IN SEDIMENT FROM THE DAY OF INSTALLATION OF THE FIELD EXPERIMENT

Saturates	Concentration (ppm)
C_{11}	13.34 ± 1.21
C_{12}	16.11 ± 1.53
C_{13}	17.79 ± 1.82
C_{14}	17.44 ± 2.39
C_{15}	19.39 ± 2.94
C_{16}	18.84 ± 3.00
C_{17}	17.03 ± 2.92
Pristane	10.74 ± 1.95
C_{18}	16.20 ± 3.22
Phytane	8.05 ± 1.43
C_{19}	18.79 ± 3.00
C_{20}	16.40 ± 2.88
C_{21}	17.56 ± 3.09
C_{22}	16.02 ± 3.37
C_{23}	15.18 ± 2.65
C_{24}	13.80 ± 2.84
C_{25}	11.71 ± 2.47
C_{26}	10.21 ± 1.97
Total saturates measured	274.61 ± 42.32

Aromatics	Concentration (ppm)
Naphthalene	7.20 ± 0.77
2-MN	14.31 ± 3.10
1-MN	10.51 ± 2.90
Total	24.82 ± 5.32
1-ethyl + 2-ethyl Naphthalene	6.93 ± 0.35
2,6 + 2,7-DMN	9.52 ± 0.75
1,3 + 1,6-DMN	9.93 ± 0.94
1,7-DMN	7.81 ± 0.64
1,4 + 2,3 + 1,5 DMN	9.14 ± 0.72
1,2-DMN	1.60 ± 0.45
Total	42.68 ± 2.74
TMN-1	3.80 ± 0.64
TMN-2	3.65 ± 0.55
TMN-3	5.69 ± 1.11
TMN-4	4.26 ± 0.76
2,3,6-TMN	2.08 ± 0.24
TMN-5	5.99 ± 0.77
Total	28.44 ± 6.10
Phenanthrene	3.05 ± 0.85
MP-1	2.74 ± 0.56
MP-2	2.34 ± 0.40
MP-3	3.94 ± 1.71
MP-4	3.49 ± 1.67
Total	12.53 ± 4.31
C_2-phenanthrene-1	7.30 ± 1.56
C_2-phenanthrene-2	2.36 ± 0.68
C_2-phenanthrene-3	2.56 ± 0.44
C_2-phenanthrene-4	2.47 ± 0.87
Total	14.70 ± 3.52
Total aromatics measured	140.35 ± 23.96

MN = Methylnaphthalene

DMN = Dimethylnaphthalene

TMN = Trimethylnapthalene

MP = Methylphenanthrene

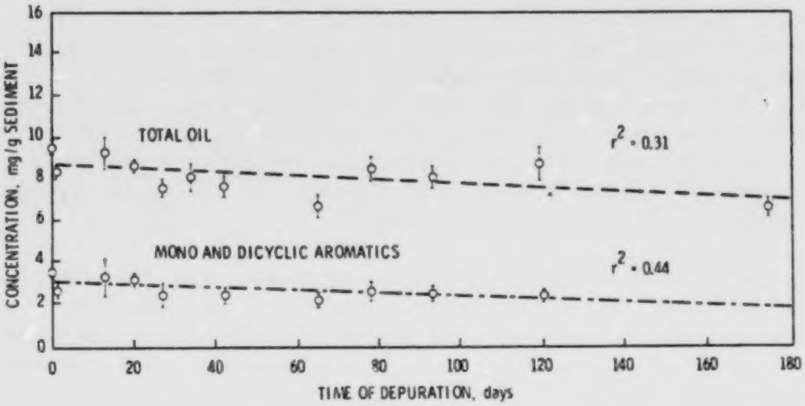
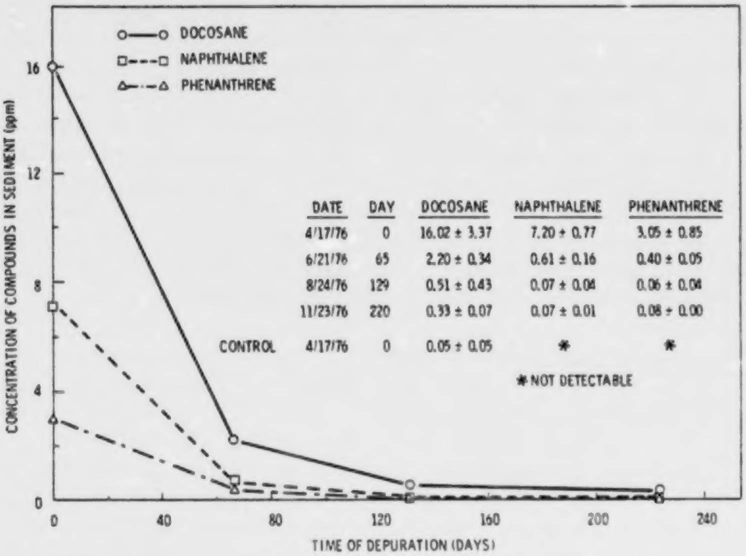


EXHIBIT 6: CONTENT OF TOTAL OIL AND AROMATIC HYDROCARBONS IN SEDIMENT AS A FUNCTION OF DEPURATION TIME



	DATE	DAY	DOCOSANE	NAPHTHALENE	PHENANTHRENE
	4/17/76	0	16.02 ± 3.37	7.20 ± 0.77	3.05 ± 0.85
	6/21/76	65	2.20 ± 0.34	0.61 ± 0.16	0.40 ± 0.05
	8/24/76	129	0.51 ± 0.43	0.07 ± 0.04	0.06 ± 0.04
	11/23/76	220	0.33 ± 0.07	0.07 ± 0.01	0.08 ± 0.00
CONTROL	4/17/76	0	0.05 ± 0.05	*	*

* NOT DETECTABLE

EXHIBIT 7: CONCENTRATIONS OF DOCOSANE, NAPHTHALENE, AND PHENANTHRENE IN SEDIMENT AS A FUNCTION OF DEPURATION TIME

(DMN) toward the elimination of N and later MN. This relationship concerning greater retention of the less soluble and probably less degradable dimethyl-isomers has been observed while animals are depurating in clean water, but not during constant long-term exposure (Anderson, 1977). While there are some small fluctuations in the levels of naphthalenes in control animals, the background concentrations resulting from absorbence of biogenic compounds in the 221-228 µm wavelength range (Neff and Anderson, 1975) are quite small.

The growth rates for these benthic amphipods are illustrated in Exhibits 8b and 8c. While the maximum length and weight levels attained by all three groups of animals were approximately equal, the control amphipods reached this size at least 3 weeks sooner. This indicates that, during early stages of the exposure, growth was significantly suppressed. Such an effect could either be the result of smaller individuals being more sensitive, or acclimation to the pollution stress taking place after a period of time. In any case, the rate of growth was definitely reduced at the 7-week interval.

Field Exposure to Oiled Sediments

Sipunculids and *Macoma* placed in oil-contaminated sediment and transferred to the field for 40 days contained significant amounts of PHC (see Exhibit 9). This is in sharp contrast to the tissue levels of the filter-feeding clam, *Protothaca staminea*. From these results, it is clear that the two deposit-feeders obtained the hydrocarbons from ingestion of sediment and not from the water column. The distribution of specific hydrocarbons within *Macoma* and *Phascolosoma* is quite similar. Triaromatic compounds (e.g., phenanthrene) and n-alkanes were found at the highest concentrations. These data give additional evidence of the significance of naphthalenes in organisms exposed to whole oil or extracts, and the di- and trimethyl-isomers were, as usual, present at higher concentrations. It is interesting to note that the parent compound (naphthalene) was absent from the tissues, as shown in earlier research (Anderson, 1977) and the amphipod experiments.

The final aspect of the biological research relates closely to the analyses of field sediments discussed above. Substrate collections have been taken at four time intervals, and there are four sets (six each) of sediment boxes in the intertidal zone. At this time, the samples of the first two collections have been sorted for biota, but only a partial list of organisms and data on the numbers of the two most prominent bivalves have been prepared. The incomplete list of species found thus far in the sediment samples is shown in Exhibit 10. As in similar benthic studies, three classes of invertebrates are well represented (Polychaeta, Mollusca, and Crustacea). A more complete picture of the number of species and individuals present in clean and contaminated sediment will be available by summer.

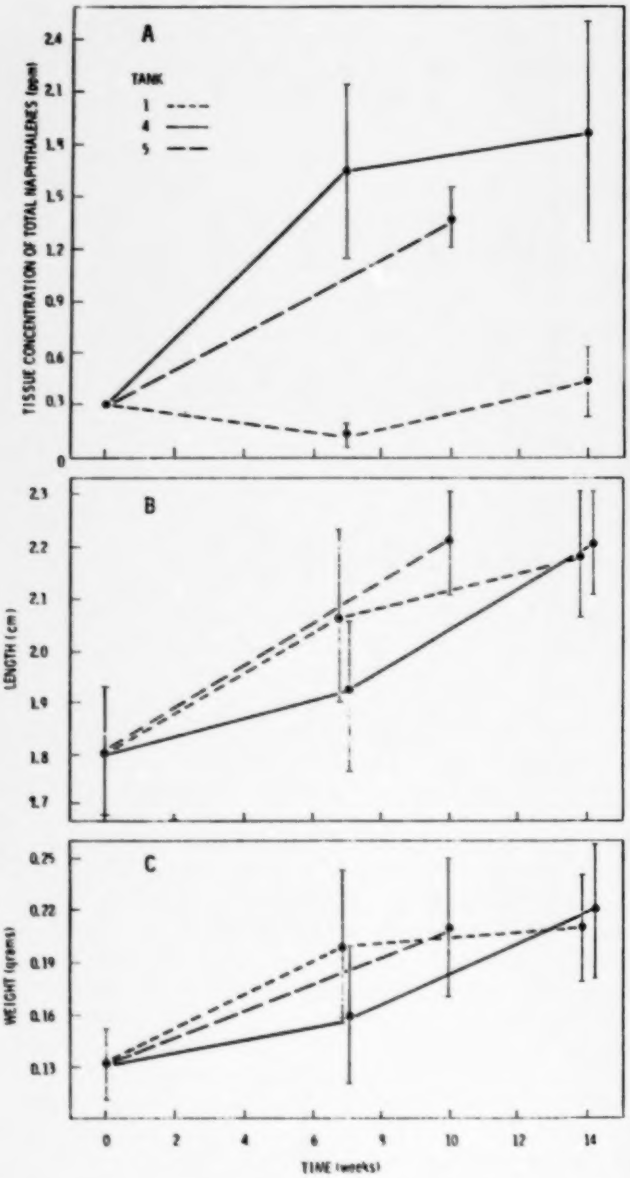


EXHIBIT 8: EFFECTS OF CONSTANT-FLOWING PRUDHOE BAY CRUDE OIL EXTRACT ON THE AMPHIPOD, ANONYX (Vertical bars represent standard deviations, where the initial sample was 20 animals and others consisted of 10 animals each, with 2 animals per naphthalenes analysis. Tanks 4 and 5 were exposed, while Tank 1 is a control. Exhibit 8a is uptake of naphthalenes; 8b is growth in length; and 8c is growth in weight.)

EXHIBIT 9: PETROLEUM HYDROCARBON CONTENT (μg/g wet; ppm) OF SIPUNCULID WORMS (PHASCOLOSOMA) AND CLAMS (MACOMA AND PROTOTHACA) EXPOSED IN THE FIELD TO OILED SEDIMENT FOR 40 DAYS
(Number in parentheses represents the number of specimens in each sample)

Hydrocarbon Content	Oil-exposed Sipunculids (3)	Oil-exposed Sipunculids (2)	Control Sipunculids (3)	Oil-exposed *Macoma* (3)	Control *Macoma* (2)	Oil-exposed *Protothaca* (2)
n-alkanes C_{12}-C_{29}	1.90	0.73	<0.10	0.69	<0.10	<0.10
1 and 2-methylnaphthalene	0.23	<0.01	<0.01	0.06	<0.01	<0.01
total dimethylnaphthalenes	0.60	0.15	<0.01	0.89	<0.01	<0.01
total trimethylnaphthalenes	0.95	0.44	<0.01	0.90	<0.01	<0.01
total triaromatics	2.25	0.77	<0.10	1.90	<0.10	<0.10
total aromatics	4.03	1.36	<0.15	3.75	<0.15	<0.15

EXHIBIT 10: LIST OF ORGANISMS COLLECTED FROM FIELD RECRUITMENT SEDIMENT BOXES

Polychaeta

Platynereis bicanaliculata
Armandia bioculata
Lepidonotus caelorus

Mollusca

(Bivalvia)

Clinocardium nuttallii
Protothaca staminea
Macoma sp.
Cooperella subdiaphana
Mytilus edulis
Mysella tumida

(Gastropoda)

Alabina sp.
Margarites sp.
Lacuna sp.
Odostomia
Bittium

Crustacea

Exosphaeroma amplicauda
Gnorimosphaeroma oregonensis
Pagurus granosimanus
Pinnixa sp.
Hemigrapsus sp.
Cumella sp.
Balanus sp.
Leptochelia sp.
Corophium sp.

The two most prominent species were the bivalves (Mollusca) *Cooperella subdiaphana* and *Mysella tumida* (see Exhibit 11). These species were found at both time intervals and in every sediment sample. Exhibit 11 not only illustrates the number of individuals of these two species, but also the ratios of *Cooperella* to the total and the level of sediment hydrocarbon contamination.

EXHIBIT 11: RELATIONSHIP BETWEEN THE HYDROCARBON CONTENT OF SEDIMENT AND THE DISTRIBUTION OF BIVALVES FROM THE SEDIMENT

	NUMBER OF BIVALVES				P P M			
Time (Days)	*Cooperella subdiaphana*	% *C.s.* of total	*Mysella tumida*	Control or Exposed	Docosane	Total* Saturates	Phenanthrene	Total* Aromatics
0	-	-	-	E	16	275	3	140
65	61	17.0	298	C	-	-	-	-
	36	18.6	157	E	2	34	0.4	18
	34	15.5	185	E				
114 †	127	17.8	588	C	-	-	-	-
	47	15.7	253	E	-	-	-	-
	170	43.4	222	E				
129	57	18.5	251	C	-	-	-	-
	54	18.3	241	E	0.5	8.6	0.06	2.8
	33	14.0	203	E				
220 **	-	-	-	E	0.3	5.1	0.08	3.7

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* Except for zero time data, these are estimates based on percentages in initial samples.

† These data are from a second field installation, which has not yet been analyzed for hydrocarbon content. The clam populations are calculated from replicate subsamples.

** Biological data are not yet available.

As discussed in the analytical section, the amounts of saturate and aromatic hydrocarbons decreased considerably by the 65th day in the field. It should be noted that the mesh bottoms on the boxes allowed natural percolation of sea water to occur as the tide level fluctuated. This process no doubt served to keep the sediment aerobic and probably increased the rates of hydrocarbon leaching and degradation. Some of the smaller organisms may have entered the boxes via the 1.6 mm openings in the mesh.

It is obvious that the sorbed hydrocarbons did not restrict recruitment by the two clam species. The numbers of individuals in oiled sediments are, in most cases, very similar to those in control boxes. The two sets of data points on day 114 deviate from all other findings. The high number of both species in control sediment is somewhat surprising, but the proportion of Cooperella to the total indicates the data are consistent with the general pattern. More difficult to explain is the sharp increase in relative proportion of Cooperella (43%) in exposed sediment, as compared with all other collections. The number of Mysella in this sample is very close to all other collections, so it would appear the the abundance of Cooperella in this oiled sediment may be because of some chemical attraction and resistance. Similar associations of Cooperella with oil-contaminated sediment have been observed earlier by Vanderhorst (unpublished data). Future biological and chemical analyses of later samples will aid in the interpretation of these findings.

DISCUSSION

The methods developed to characterize the hydrocarbon components in the flowing sea water exposure system have been shown to be sensitive and accurate. In addition, the analytical results demonstrated that the delivery system produces a consistent level of contamination in the exposure tanks. When the methods used to analyze Prudhoe Bay crude oil for component hydrocarbons (gas capillary chromatography) were applied to both fine and coarse sediments, the separation of specific compounds was extremely good.

On the basis of the results generated thus far, the experiments have been useful in demonstrating the differences in the potential persistence of crude oil utilizing undisturbed fine-textured marine sediments under anaerobic laboratory conditions compared to a field experiment using coarse beach sediment under aerobic conditions. Furthermore, these experiments have provided sets of samples for development of analytical techniques appropriate not only for use in conjunction with related biological studies of oil-contaminated sediments, but also to studies involving the uptake and metabolism of oil by marine organisms. To cite an example, we are currently developing HPLC techniques to be used alone and with our existing capillary chromatography methods to replace conventional column methods. These analytical techniques, coupled with the appropriate biological experiments, will provide much needed information on the chemical, physiological, and community effects that would be observed for marine organisms exposed to these types of experimental conditions.

It was found that the flowing exposure system delivered from 0.22 to 0.24 ppm of total hydrocarbons (IR), and that level slowed the growth of Anonyx during the first 7 weeks of exposure. Anderson and Kiessler have compiled an extensive amount of unpublished data on this species, and some aspects of these findings can be used for comparisons.

In static bioassays using sea water extracts of Prudhoe Bay crude, an initial concentration of about 6 ppm is required to produce the 96-hr LC_{50}. Animals dying during exposure contain from 5 to 11 ppm total naphthalenes, while those surviving at lower concentrations contain from 0.9 to 1.8 ppm total naphthalenes. The tissue concentration of amphipods exposed for 14 weeks at very low concentration (0.23 ppm) was 1.8 ppm total naphthalenes, which indicates that our exposure is simulating chronic contamination which is very near the threshold of tolerance. Further evidence of this is the marked reduction in production of viable offspring by gravid females, which we observed in mid-February 1977. In other words, long-term exposure to a concentration which is 0.04 times the static system 96-hr LC_{50} value has reduced growth of adults during the early stages of exposure but not inhibited maximal growth. The final effect of this exposure, which is most meaningful, is the inhibition of reproduction; however, this was not observed until 19 to 20 weeks after initiation of the experiment.

To our knowledge, there have only been two previous reports of hydrocarbon uptake from sediment by deposit-feeding marine organisms. Anderson et al. (1977) described the rates and extent of naphthalenes uptake by the sipunculid (Phascolosoma) exposed to sediment that had either been mixed with Prudhoe Bay crude or had received a layer of oil on the surface. These animals did contain contamination after 2 to 14 days of laboratory exposure, but the levels approximated that in the sediment, and depuration of 6 to 14 days resulted in almost complete loss of naphthalenes. Rossi and Anderson (1977) exposed the polychaete (Neanthes arenaceodentata) to sediment containing 9 ppm total naphthalenes for 28 days, and the animals never exhibited levels above background. When these worms were fed artificial detritus containing about 10 ppm ^{14}C-2-methylnaphthalene, they found the accumulated ^{14}C-activity was voided in the feces during 24 hr with clean food and water. Roesijadi et al. (unpublished) reported on the uptake of naphthalenes by the detritus-feeder, Macoma inquinata. The contamination that was present in these clams apparently came from small amounts released to the water and not from the ingested detritus.

This paper, for the first time, presents data on the uptake of numerous PHCs by three species present in contaminated sediment held for a long period (40 days) in the natural environment. Table 9 demonstrated the extent of hydrocarbon uptake by the two deposit-feeders, in contrast to the lack of contamination in Protothaca, which is a filter-feeder. This is probably the most significant approach to determine uptake of hydrocarbons via ingested sediment particles, as all three species were surrounded by oiled substrate. While n-alkanes were present in Macoma and Phascolosoma at significant levels, the aromatics, and particularly the triaromatics, were found at rather high concentrations. This approach will be continued to determine the rate and extent of uptake, as well as the specific compounds of significance. These preliminary findings demonstrate that benthic deposit-feeders take up large molecular weight aromatic compounds from polluted sediment. These hydrocarbons are the most harmful to marine organisms, as well as vertebrates, including man.

The final aspect of this research concerns the natural rates of hydrocarbon depuration from oiled sediment and the effects of these compounds on the recruitment of benthic invertebrates. The significance of the analytical approaches and findings have already been noted, and without them the biological data would be meaningless. The only other investigation regarding correlations between hydrocarbon content in sediment and the distribution of benthic organisms is as yet unpublished (Armstrong et al.). For more than 1 year, these researchers have recorded the concentration of naphthalenes in the sediments near an effluent of an oil-separator platform in Trinity Bay, Texas. Along three transects projecting away from the source of contamination, they have recorded the numbers of individuals and species in sediment samples and compared these to the concentrations of naphthalenes present. Stations within 500 ft of the platform had depressed benthic faunas, and there was a correlation between levels of sediment naphthalenes and reduced populations of organisms. A peak ("stimulation zone") in both numbers of species and individuals was found at between 2,250 and 5,500 ft from the platform.

While sufficient time has not been available to complete either chemical or biological analyses of all samples collected thus far, we have not seen an effect on recruitment of benthic bivalves. As the hydrocarbon analyses show rather rapid loss from field sediments, the levels could have decreased to the point at which summer larvae were not inhibited from settling. There are no apparent effects on the two clam species inhabiting these sediments for approximately 3 months. Only by continuing analyses of later collections and installing new contaminated sediments, can these questions be answered.

It should be noted that this research is ongoing, and the results presented in this paper should be considered preliminary. Thus far, the findings clearly indicate that the approaches and techniques being utilized have been productive in deriving much needed information on the fate and effects of petroleum hydrocarbons in the marine environment. Each aspect of the research has and will provide sufficient data to warrant publication separately.

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DISCUSSION

Dr. Spies: With respect to the movement of sediment that might have occurred, could you describe how that was broken down?

Dr. Anderson: We still see the dominant contamination in the top layer. This material does not move readily. Since it is not an area where there is active movement, we recently mixed in glass beads coated with barium that can be analyzed to ensure that this material is not being replaced by other clean sediment.

From the floor: You show a marked difference between field weathering and laboratory conditions. How would you prepare a weathered fraction test?

Dr. Anderson: We have this large treatment facility with a floating layer of oil on top of it. The oil builds up to the point where it must be pumped off. We have considered using that as our weathered oil. However, it would not be very good since it contains a few other toxicants from our other systems. A sharp difference in the toxicity of the water soluble fraction results just from putting an aerator in a soluble extract.

Dr. Dieter: In your exposure for the clams, you showed about 4 ppm accumulated.

Dr. Anderson: That was the total analyzed. The oiled sediments, the clams, for instance, contained about 600 ppm of total hydrocarbons to simulate a real spill situation. So that is not a magnification, by any means. This is why I use the word "uptake," rather than "accumulation," because you can talk about a large accumulation from water. From sediment, you are usually trying to discover if there is significant uptake. Over time, there was a large loss of this material.

Dr. Davies: Have you considered the fact that aksane and carbon tetrachloride may not be able to extract some of the materials?

Dr. Riley: As far as the carbon tetrachloride extractions are concerned, we have proved that you can get approximately 97% recovery of the oil from the sediments. Currently, we have an ongoing experiment to verify a similar type of recovery from the hexane extract. The techniques are essentially the same except for the solvent, and we expect that the recovery will be about the same.

Dr. Anderson: The hexane in tissue extract is not nearly as efficient as, for instance, diethyl ether. We always tend to test these to see what kind of efficiency we are getting. Using a resin column system, we have tried to see if we are collecting all the petroleum and getting the efficiency of extraction. However, we are working with relatively high amounts so it is not a problem. If we get down to very low levels, it might be a problem.

Dr. Davies: In our laboratory, some experiments with pesticides have shown that some of it is getting bound in the salmon and it is then extracted. So, we are doing tracer experiments.

Dr. Lefcort: Are you planning any histological examination of the field-exposed bivalves? We are interested in the neoplasia that occurs in these.

Dr. Anderson: We have proposed from time to time to do some work with the flat fish in the area. In fact, I know of some similar ongoing experiments in the NOAA laboratory. Frankly, our laboratory is not geared up for histological examinations.

Dr. Baumgartner: Do you have any other data on interstitial analyses in the laboratory sediments to give you any indication?

Dr. Anderson: It is obvious underneath a film that is perhaps only ½ in. thick. You can see the sharp break between the upper tan zone and the black anaerobic mud beneath. There really is no doubt about it. However, the chemical measurements have not been done.

Dr. Baumgartner: Where is the oil sample? When you take these degradation samples, do you mix a vertical column?

Dr. Anderson: With that particular mud system, we take just a scoop and obtain surface plus 4 or 5 cm beneath. In the field sites, we are using PBC cores to take samples.

Dr. Kunhalt: You mentioned the reproduction of some of your test animals. Which species were they and what did you find about egg numbers or hatching?

Dr. Anderson: We do not have all the data yet because they are still hatching. We have looked at the brood pouches of one species of anthropod that was constantly exposed for 19 to 20 weeks in the system. In many of these, the eggs were not completely developed, whereas, by now, most of the controls have hatched out completely. The number of offspring seems to be reduced, but we will not have good data on that for awhile. The number living of the juveniles that hatch is dramatically different. We essentially have not seen any living juveniles from those females, whereas all the juveniles that hatch from the controls are living.

Dr. Kunhalt: Were these the animals that did not show any difference in growth until maturation?

Dr. Anderson: That is right. The last growth point, however, was 14 weeks, which was before we saw the brood pouch development of the females, so we did not include the females. Essentially, that was non-gravid growth.

Dr. Kunhalt: What size are they? Could they be analyzed by normal methods to get accumulation in the eggs?

Dr. Anderson: Oh, yes. We have some other ongoing studies with shorter term exposures, and about four different ongoing experiments with gravid female anthropods.

ENVIRONMENTAL ASSESSMENT OF NORTHERN PUGET SOUND AND THE STRAIT OF JUAN DE FUCA

Howard Harris*

The environmental assessment of Northern Puget Sound and the Strait of Juan de Fuca is a 5-year study that addresses the potential marine environmental and ecological consequences of the intensified petroleum activities anticipated for the next decade. It is one of a series of projects funded by the EPA that will provide the information needed to identify and cope with the impact of accelerated energy development. This research is administered by National Oceanographic and Atmospheric Administration's (NOAA) MESA (Marine Ecosystems Analysis) Puget Sound Project Office in Seattle, Washington.

REGIONAL OVERVIEW

The Puget Sound region (see Exhibit 1) has never been a busy petroleum center, as it has imported and refined only enough crude oil to supply its local population. Since most of the crude has been supplied via Canadian pipeline, there has historically been only minor shipping of petroleum and petroleum products through the area's waterways. Recently, however, the amount of tanker traffic has increased, and a further intensification of petroleum-related activity is anticipated for the coming decade. Two main factors account for this increase: new suppliers and a potentially larger market.

Canada has announced that it will cut off all shipments of crude oil to the United States by 1981. The Federal Energy Administration (FEA) has determined that oil delivered in the interim should be transported directly to the Midwest, which means that all Canadian oil to the Seattle region will cease flowing in 1977. Not only does this action require supplying the Northwest via tanker, but it also results occasionally in a surplus of crude oil in western Canada. This surplus is shipped out from terminals near Vancouver through the Strait of Juan de Fuca.

The second factor is possible expansion of local petroleum facilities to serve other marketing areas. Puget Sound is the closest U.S. port to the termination of the Alaskan pipeline. Its natural

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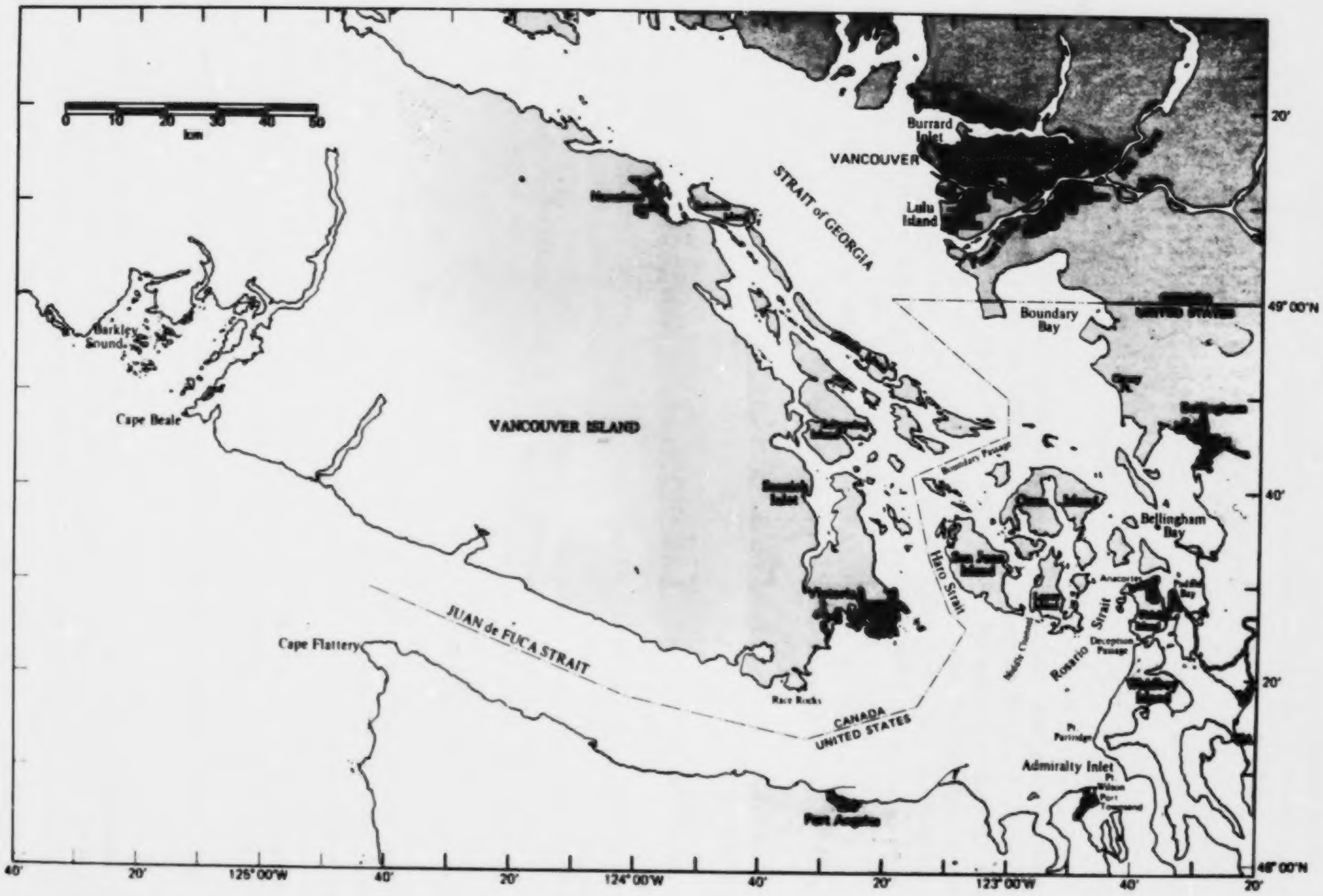

EXHIBIT 1: STUDY AREA

deep harbors make it ideally suited to serve as a major transshipment point for other regions of the United States. Under this scheme, crude oil would be shipped into the region via tanker and either piped out to the Midwest, refined and shipped to California, or both. The Oceanographic Institute of Washington, in a study for the state legislature, has evaluated several scenarios and has estimated that Puget Sound tanker traffic could increase tenfold by the end of the century. No pipeline construction is yet underway, and plans for this type of expansion remain incomplete, although several area refineries presently plan construction of deepwater ports to accommodate larger vessels.

Since tanker routes to the existing refineries at Cherry Point and Anacortes traverse major fishery and recreational areas of the Strait of Juan de Fuca and northern Puget Sound, much local publicity has been given to the threat of oil pollution to marine, economic, recreational, and aesthetic interests.

The Washington State Legislature has recognized the need to formulate a realistic energy policy and has taken several steps to prevent radical departures from the current petroleum transport methods. One of these steps was a moratorium on construction of new marine oil transfer facilities; another was the Tug Escort Law, which severely limits the movement of supertankers in waters east of Dungeness. (The Tug Escort Law has been successfully challenged in court as unconstitutional by the Atlantic Richfield Company.)

In 1974, the Washington Energy Policy Council appointed by the governor recommended that area refineries should not be expanded to serve more than the traditional marketing areas, and that the existing refineries should all be served by a common-use terminal in or west of Port Angeles. This recommendation was endorsed by the then-Governor Evans, and the Port Angeles common-use terminal is under heavy study by all concerned parties. In January 1977, Governor Evans was replaced by Governor Ray. At this time, the question of state energy policy is unresolved, but it is being actively addressed by the new governor and by the state legislature.

In response to this situation, a number of investigations have been initiated to provide a basis for rational decisions on the management of Puget Sound. These include both investigations focusing upon the existing refinery sites at Cherry Point and Anacortes (and the possible transfer site at Port Angeles) and laboratory investigations concerned with effects of low concentrations of petroleum upon the native biota.

The State of Washington Department of Ecology (DOE) has initiated baseline studies in the high-risk region near the existing refineries at Cherry Point and Anacortes and in the San Juan Islands. The program was initiated in 1973 and is funded at least through a second biennium to June 1977. The main accomplishments so far include: a survey of the intertidal and shallow subtidal biological resources through a habitat-specific analysis of community structure; publication of a literature review on the region's biologic resources; and development of an economic value index of the resources that might be affected by oil pollution.

Battelle Northwest has been funded by the U.S. Energy Research and Development Administration (ERDA) and EPA to investigate the potential effects of long-term exposure to petroleum-derived hydrocarbons through laboratory studies and in situ exposure of selected communities. Battelle Northwest has also undertaken an extensive analysis of petroleum contaminants in water, organisms, and sediments near Cherry Point, Anacortes, and Port Angeles.

The University of Washington is performing a multiyear sampling program to determine the distribution of aliphatic, aromatic, and sulfur-containing molecules in organisms, sediment, and water at stations in Puget Sound and along the Washington coast. The sources and food web transfer of these compounds are under investigation.

EPA is funding NOAA to investigate the fate and effects of petroleum hydrocarbons and selected toxic metals in marine ecosystems and organisms. Although this research is not specific to Puget Sound, a part of the study will be conducted in this area or in Alaska and should contribute directly to the understanding of potential effects in Puget Sound.

RESEARCH PROGRAM

The goal of this research program is to determine the principal regional characteristics that must be known to assess the potential environmental effects of increased tanker traffic, petroleum transfer operations, and refinery capacity within northern Puget Sound and the Strait of Juan de Fuca. It is anticipated that 5 years will be required to complete this program. Additional research, not encompassed by this program (e.g., effects of hydrocarbons upon the biota), must be performed by others before the total implications of increased concentrations of petroleum within the environment can be assessed.

High priority is given to documentation of the present status of the marine ecosystem, which presumably is little contaminated by petroleum or other substances. Information needed to deal with a massive oil spill is also given high research priority, since resultant ecological effects would be immediately and widely present. Factors related to chronic emissions are given a lower priority. The scope and depth of research are determined in part by the constraints of available funding and resources, as well as by the need to perform certain tasks before the movement of Alaskan oil into Puget Sound in 1977.

The research is directed toward four major objectives:

1. Characterize the major marine biological populations subject to pollution impacts resulting from petroleum transportation and refining

2. Determine the existing distribution and concentration of pollutants within the ecosystem that are associated with refinery effluent and petroleum

3. Characterize the principal processes and major pathways by which petroleum moves through the marine ecosystem

4. Provide decision makers with environmental and ecological information and predictions of the effects of oil-related activities upon the ecosystem.

A series of tasks leading to the accomplishment of these objectives have been defined in the project development plan for this assessment dated September 1975. The output from each task will furnish necessary, and otherwise unavailable, information. These tasks are coordinated with other research to avoid duplication of effort.

The general approach is as follows:

- The biological populations to be investigated include the intertidal and deep benthic invertebrates, nearshore fishes, marine mammals, and marine and shoreline birds. These populations will be characterized according to their composition, relative abundance of species, trophic relationships, and seasonal and spatial distribution.

- The present concentrations of selected hydrocarbons and other constituents of petroleum will be determined in water, sediment, and representative animals. The majority of samples will be collected from the intertidal region, but some attention will be given to midchannel sampling.

- The major water mass circulation patterns will be determined with emphasis upon the surface layer. The contributions of tides, estuarine flow, and winds will be evaluated. More detailed, localized investigations will be undertaken near existing or potential marine terminal sites. Data will be incorporated into a numerical model developed to predict the movement of spilled oil. Other investigations will evaluate the capability of the microbial populations to degrade oil and the capacity of naturally occurring suspended materials to scavenge hydrocarbons from the water column.

- Available knowledge in selected topical areas will be summarized and made immediately available to local decision makers in appropriate formats.

Ultimately, the results of this research will be summarized and interrelated with data from other petroleum studies to characterize the major components and processes of the region's ecosystem, the transport and fate of spilled petroleum, and the biologic response to oil pollution.

The principal planned users of the study results are planners and decision makers at all government levels concerned with the changing nature of the petroleum industry in Puget Sound, including:

- A change in crude oil supply from overland pipeline to tanker
- A possible expansion of the traditional marketing area and quantities handled to include transshipment of crude oil to the Midwest or refined products to California
- Construction of a common-use terminal at Port Angeles or Burrows Bay to handle supertankers in lieu of expansion at the existing refineries
- Modifications and/or expansion of the contingency plan to accommodate the changing situation.

Decisions on these and other questions require detailed knowledge of the marine ecosystem; this knowlege is largely lacking at present.

Additional users include the petroleum industry and coastal zone managers in general.

FIRST-YEAR RESULTS

Since this research represented a new and major undertaking, a project development plan was first prepared and approved. This plan details the thrust of the research program and the specific tasks to be accomplished. In late 1975, arrangements were made with a number of principal investigators, and field work began early in 1976. At the present time, results from the first year are becoming available on the biological, petroleum baseline, and physical oceanographic program and model predictions.

Biological Program

The biological program has focused initially on three communities particularly susceptible to damage from floating oil: the intertidal biota, the nearshore fishes, and the plankton. Geographically, the field programs have concentrated on the Strait of Juan de Fuca, since information on this area is needed but lacking and since complementary information on the Cherry Point-Anacortes-San Juan Island region was being collected by contractors for DOE. The DOE data have been obtained and entered into a common data file with the results of this research.

Plankton investigations have emphasized ichthyoplankton and zooplankton, with less attention to phytoplankton. Samples have been collected a total of seven times during 1976, about every 6 weeks except during midwinter. A total of nine stations is occupied: three across the mouth of the Strait, three in mid-Strait, and three near

Port Angeles. A report on the 1976 work has been prepared by the principal investigators. There is evidence of distinct spatial and temporal trends in community composition and relative abundance, reflecting both the oceanic influence and that of Puget Sound.

The distribution and abundance of intertidal and shallow subtidal organisms are being determined at 10 sites along the Strait of Juan de Fuca. A stratified random sampling scheme is being used with quarterly sampling frequency. Analyses of the spring and summer samples are essentially complete. Based upon preliminary analyses, the biota is extremely rich and varied, compared with the San Juan Islands and Puget Sound. Many species that were previously undescribed have been found. Community characteristics vary greatly with substrate type and season.

At six of the intertidal stations, nearshore fishes are collected with a beach seine and a tow net. Captured fish are identified, counted, weighed, and their stomach contents analyzed to determine predator-prey relationships. Preliminary analysis of the first three collections has disclosed spatial and temporal patterns in predator-prey relationships and community composition. Prey communities found in the fish stomachs have not yet been compared with those of the intertidal-shallow subtidal benthic communities.

Petroleum Baseline Program

The Puget Sound ecosystem has not been subjected to exposure to massive amounts of petroleum. Concentrations of hydrocarbons are presumed to be very low, although data to substantiate this contention do not exist. Baseline levels will be determined throughout the region, both in close proximity to existing petroleum transfer facilities and at remote locations.

During 1976, preparations were made to conduct the baseline survey. Funds otherwise provided by EPA were used to establish the NOAA National Analytical Facility at the Northwest and Alaska Fisheries Center (NWAFC) in Seattle. Concurrently, this program supported the NWAFC in the development of specifications for the baseline study. This involved two workshops attended by about two dozen experts in various disciplines; identification and testing of various field and laboratory procedures; and preparation of a summary report. Determination of baseline concentrations is beginning in February 1977 at 19 sites. At each site, duplicate composite samples of mussels and of intertidal sediment will be collected on a quarterly basis. Analyses will include total aliphatics, each n-alkane from C_{14} to C_{31}, total aromatics, and 17 specified aromatics.

Physical Oceanographic Program

The oceanographic program began in February-April 1976 with the deployment of current meter arrays in the Strait of Juan de Fuca near Port Angeles and wind recorders at strategic locations throughout the region. Three subsurface arrays with four Aanderaa meters each were deployed across the channel, one at the midpoint and one near each shore. An air-sea interaction buoy with current meters was also deployed near the central mooring to investigate in detail the oceanographic response to winds.

With the arrival of additional equipment, a more ambitious deployment was undertaken during the November-February period to investigate the ocean/wind processes typical of winter conditions. Five subsurface current meter arrays were deployed in a cross-Strait transect off Pillar Point and one in midchannel off Cape Flattery. Three air-sea interaction buoys were also deployed: one in mid-channel off Cape Flattery, one off Pillar Point (in mid-Strait), and one off Tongue Point (west of Port Angeles). In addition, five special wind meters were deployed around the Strait to augment the permanent stations, and six wind recorders were deployed around the Olympic Peninsula to measure the larger scale wind patterns.

Data from the February-April deployment have been processed, and analysis is underway. Processing is beginning on the records from the November-February deployment.

In another study, the seasonal and spatial occurrence of suspended solids is being determined as well as their sorptive characteristics relative to selected petroleum constituents. Thirty-three stations in the eastern Strait and northern Puget Sound were occupied in November for the purpose of making nephelometer profiles, collecting filter samples, and collecting 4 g size samples for later laboratory analysis.

Information Dissemination and Model Predictions

The principal vehicle for dissemination will be a series of interpretive monographs on various aspects of the Puget Sound ecosystem/environment. During 1976, the specifications for the series were prepared, based upon examination of comparable series for other geographical areas, local workshops, and responses to a questionnaire. There appears to be a large demand for a series with a level of descriptive writing about equal to Scientific American with emphasis on graphical presentations.

The 1971 annotated bibliography of the Puget Sound marine environment is being updated and will be available in March 1977. Content is being expanded to include a more elaborate indexing system, entries for the lower Strait of Georgia, and coverage of coastal zone management and related topics.

A report has been prepared on the petroleum industry in Puget Sound. Included is information on crude inputs according to types, amounts and sources, refinery processes and wastewater characteristics and product outputs. Copyright releases are being sought, and the report should be available this spring.

Mathematical model development began in 1976 with the adaptation of a general oil spill trajectory model developed for the Bureau of Land Management in Alaska to the Puget Sound region. Work is proceeding on various required submodels, e.g., a meteorological submodel to give surface winds, a tidal current submodel, and spreading submodel. Results of field investigations are being used to develop and/or verify model predictions.

A study was completed in 1976 to assist in defining a strategy for possible development of additional models. The study described the potential role of models in providing solutions to questions by state and local governmental management about marine environmental impacts that could result from alternative petroleum developments. Both literature review and personal interviews with officials from state and local governments were employed. A list of management questions and impacts was developed, and the types of models that would be appropriate to the solution of these questions were identified.

FUTURE DIRECTIONS

This assessment is now in the second year of a 5-year research program. At the end of this period, the major components of the ecosystem will have been described and quantified. Other related investigations in Puget Sound are on a similar schedule. The vast majority of this research is being funded by the federal government.

It is apparent that an opportunity will exist beginning about 1980 to synthesize the output from these investigations and to deal in a more sophisticated manner with the consequences of energy development. Perhaps it is as much an obligation as an opportunity. A large investment of public funds will have been made in this research, and some attention must be given to ensuring that commensurate benefits are obtained.

Long-term priorities and requirements in marine research relevant to energy development are topics to be consided later in this meeting. These same topics regarding petroleum and Puget Sound are also being addressed within NOAA, as plans for the MESA Puget Sound Project are being developed for the early 1980s. A continuing opportunity for cooperative research exists and should be pursued at the appropriate time.

DISCUSSION

Dr. McErlean: I was curious to know whether you are involved in any of the 208 planning that area.

Dr. Harris: No, we are not. The 208 planning in that area, as I understand it, has largely been on the land side, and without too much consideration given to the marine aspects.

Dr. McErlean: Is it just being approached from the land point of view?

Dr. Harris: I believe, at this point, that 208 and coastal zone management are proceeding rather independently. The State of Washington has been involved in coastal zone management or shorelines management, as they call it, for a number of years. In fact, their plans have existed for almost 3 years, although they were just recently approved at the federal level.

Dr. McErlean: It might seem that the data you have might be of benefit to the 208 agencies.

Dr. Harris: Of course, the data are available. However, I am only aware of the 208 planning activities in one-half of the water body off of Seattle. They are not in the area of the Strait of Juan de Fuca, where we are working.

Dr. Morgan: Is there any reason that the biological or hydrographical surveys have not considered the movements of the deep sediments in the Strait of Juan de Fuca? You may be looking at plankton, but not the deep sediments.

Dr. Harris: Later on, we will do some work with the deeper benthic out in the Strait of Juan de Fuca. To digress, the Bureau of Land Management recently held a conference in Portland to discuss the potential lease sites off the coasts of Oregon and Washington, and one session dealt with Puget Sound. The participants were very concerned about the deeper parts of the Strait of Juan de Fuca. They felt that offshore development could well have its greatest impact on the strait. I am not sure that we can address that adequately within this program.

ENVIRONMENTAL ASSESSMENT OF AN OFFSHORE OIL FIELD: A PROGRESS REPORT

Richard J. Berry and Ernest D. McRae*

Federal research related to offshore petroleum development has expanded rapidly in recent years because of the legal requirement to consider possible environmental consequences. As part of this effort, the National Oceanographic and Atmospheric Administration (NOAA) initiated a 5-year assessment of an active oil and gas field through its component agency, the National Marine Fisheries Service (NMFS). In 1975, responsibility for management and implementation of appropriate studies was assigned to the NMFS laboratory in Galveston, Texas.

The area selected for study is the Buccaneer Oil Field located about 53 km (32 miles) south of Galveston (see Exhibit 1). This field was chosen because it is isolated from other production areas and has been in operation long enough to allow development of climax communities. Situated in commercial shrimping grounds, it is a focal point for recreational fishing and diving activities. Objectives of the study are to compare ecosystems in the vicinity of a producing field with those in nearby unaltered areas and to identify changes attributable to pollutants and the presence of structures.

DEVELOPMENT OF THE BUCCANEER FIELD

Seismic surveys in the Buccaneer Field area were conducted from 1953 through 1959, and Shell Oil Company acquired some 43,000 acres during the federal lease sale in February 1960. An exploratory well was drilled in November 1960 to a depth of 16,088 ft, but it was subsequently abandoned because of mechanical problems. Additional seismic surveys were performed during 1961-1962 to assess the size, shape, and complexity of the structural trap. A second well was drilled in October 1963, and 21 additional wells were drilled within the year. The "A" production platform was erected in September 1964, and 15 wells were drilled from it during the period from January to September 1965. Platform "B" was installed in May 1965, and 15 wells were drilled from it

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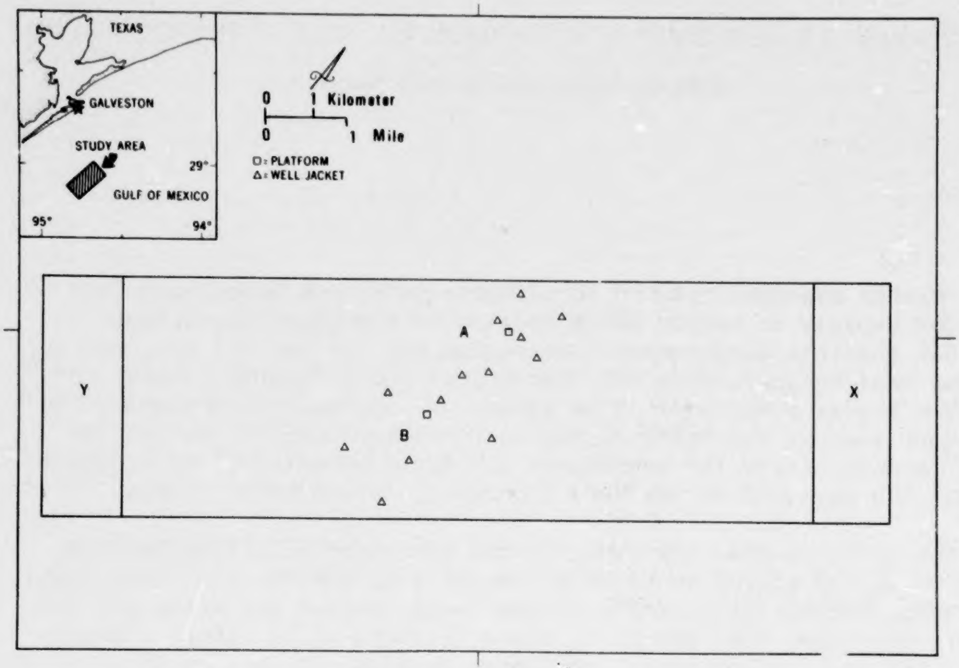

EXHIBIT 1: LOCATION OF THE BUCCANEER FIELD STUDY AREA AND STRUCTURES WITHIN THE FIELD (A and B indicate production platforms, and X and Y designate central areas to be compared with the field.)

between October 1965 and July 1966. More wells were drilled between 1966 and 1971, and additional acreage was acquired in the May 1968 lease sale. Through June 1971, 81 wells were drilled, 56 of which were completed and the remainder plugged. Currently, there are 34 active wells, including 31 gas and 3 oil completions. As the field developed, an onshore receiving plant was constructed near Freeport, Texas, and connected to the field (Platform A) by a 20 in. diameter flowline, 43 miles in length.

At present, structures in the Buccaneer Field include two production platforms (A and B), two platforms attached to A and B that serve as living quarters, 13 satellite well platforms, flare stacks, and pipelines. Only 6 of the 13 satellite platforms are currently active. Satellite platforms or jackets have helicopter pads measuring about 20 ft x 20 ft and are supported by four legs fitted around well caisons. Underwater pipelines connect the satellite wells to a production platform. Once drilling is completed, no gas or oil is stored or discharged at the satellite structures.

Each of the two production platforms have two decks about 150 ft square and are supported by legs or piles. Oil and gas from wells associated with the production and satellite platforms flow into high pressure vessels on the lower decks. These vessels separate the gas from oil and water. All gas from the field is compressed at Platform B, piped to A, and then to the Freeport facility. The oil and water flow to pressure-step-down vessels, where the two are separated by gravity. Oil is piped to storage tanks on Platform A, prior to being sent by pipeline to Freeport. Produced water flows to skim tanks, where remaining petroleum products are separated and returned to the oil stream. The water is discharged overboard at rates of 600 to 900 barrels per day at each production platform. About 3 barrels of water are produced for every 2 barrels of crude oil and condensates.

Each quarter's platform includes living facilities, a gas-powered generator, desalinization plant, sewage treatment plant, and a helicopter pad. Materials dumped into the sea are limited to edible food scraps, treated effluent from the sewage plants, and salt water. Solid wastes are transported to shore. Flare stacks associated with production plants are emergency safety devices, which normally have no discharges. No major oil spills have been reported from the Buccaneer Field. Five minor spills between September 1973 and September 1975 amounted to a total of about 6 barrels.

STUDY PLANS

A pilot study was conducted during November and December 1975 to determine the suitability of the Buccaneer Field for the proposed investigations. Bottom grab samples were obtained from 34 stations in and near the field. Analyses included classification of sediments, enumeration of infauna, and measurements of hydrocarbons and trace metals in the sediments. Notable findings were that:

1. Sediments are generally poorly sorted, fine-grained, silty sands

2. Benthic populations were essentially similar except at one station near Platform A, where they were reduced

3. Hydrocarbon levels in sediments ranged from 0.006 to 0.530 ppm with the exception of the station near Platform A, which had a concentration of 306 ppm

4. Greatly elevated levels of zinc (313 ppm) and lead (41.5 ppm) were noted at the same platform station.

Direct evidence of pollution related to oil production was found only at the one location and no residual effects of drilling activities were apparent.

Detailed plans for the first year of field work were developed from information gained during the pilot study, and contracts were awarded in April 1976. Elements of the study were divided among the NMFS laboratories at Galveston and Panama City, Florida, and four Texas universities (see Exhibit 2). Responsibility for data management was given to the NMFS laboratory, Bay St. Louis, Mississippi. All project data are forwarded to the Environmental Data Service, NOAA, for permanent storage.

EXHIBIT 2: LIST OF STUDY ELEMENTS, PRINCIPAL INVESTIGATORS, AND CONTRACTORS DURING THE FIRST YEAR OF RESEARCH AT THE BUCCANEER FIELD

Study Element	Principal Investigator	Contractor
Pilot Study	Dr. D. Harper	Texas A&M University
Data Management	Mr. P.C. Cook	NMFS, Bay St. Louis
Geology	Dr. J. Anderson	Rice University
Benthic Fauna	Dr. D. Harper	Texas A&M University
Demersal Fish	Mr. K. N. Baxter	NMFS, Galveston
Ichthyoplankton	Mr. J. Finucane	NMFS, Panama City
Pelagic Fish	Dr. L. Trent	NMFS, Panama City
Rig Fauna	Dr. N. Fotheringham	University of Houston
Hydrography	Mr. J. Martin	NMFS, Galveston
Hydrocarbons	Dr. B. Middleditch	University of Houston
Trace Metals	Dr. R. Schwarzer	Rice University
Total Organics	Dr. W. Behrens	University of Texas
Modeling	Dr. B. Gallaway	LGL, Limited

To simplify discussion of the study elements, they are grouped into benthic, water column, pollutant, and modeling components. It should be emphasized that information developed to date is of a preliminary nature and represents only 6 to 8 months of field activities. Few substantive conclusions can be drawn at this time.

BENTHIC STUDIES

The basic study plan is to compare conditions and fauna in the oil field to nearby control areas, which presumably are similar but unaffected by either the structures or pollutants. Exhibit 1 illustrates the relative positions of the oil field and two control areas (X and Y) located in similar water depths (17-23 m) and about 8 km (5 miles) from the field. Benthic stations were established on lines radiating from platforms A and B to detect effects attributable to these structures and at other arbitrary positions throughout the study area.

Geologic Studies

Geologic studies included magnetic profiling to locate pipelines, a sidescan sonar survey to detect irregularities in the seafloor surface, a seismic survey to delineate near-surface structures, and coring to obtain sediment samples. The magnetic survey proved to be a practical means for establishing the present position of pipelines, except in the vicinity of platforms where the concentration of steel structures prevented interpretation of data. Many of the pipelines had moved several meters since they were originally set in place. Pipelines resting on the bottom were also visible in records produced by sidescan sonar. The seismic survey was intended to determine near-surface geological structures. Resolution of signals was adequate for penetrations of 40 to 75 m. Evidence of subsurface structural relief between 53 and 55 m indicates the presence of a salt dome beneath the oil field platforms and an old but inactive fault east of the field.

Sediment samples were obtained from 94 stations (see Exhibit 3), using a large corer (15 cm in diameter) in areas away from pipelines and a small, lightweight corer near the lines. X-radiographs of cores revealed a little by way of sedimentary structure, because of apparent mixing by benthic organisms. The character of sediments is variable and suggests high energy bottom currents in the study area. The origin of various sediment types is still uncertain, but areas of shell and clay may represent ancient deposits exposed by bottom currents or structural uplift. Sedimentary data suggest bottom scouring within 100 m of platforms that may operate to disperse materials deposited during drilling.

Benthic Fauna Analyses

Because of the danger of rupturing pipelines with heavy gear, benthic samples were obtained by divers pushing Ekman grabs into the sediment. Triplicate collections were made at 48 stations in July 1976, and about the same number are to be obtained during three other seasons. Subsamples were removed from each grab with plastic tubes for counts of meiofauna. Station locations correspond to the sediment stations radiating from platforms A and B. Counts of macrofauna from July collections indicated depressed populations in the immediate vicinity of the production platforms and possible zones of higher than average numbers at distances of 500 to 1,500 m. Differential distributions of various forms make further interpretations difficult. Polychaetes are the dominant form (50-86% of the total), followed by lesser numbers of crustaceans and mollusks. Meiofauna counts also appear to decrease in the vicinity of production platforms, but exceptions occur. Nematodes and foraminifera comprise larger proportions of populations near Platform A than elsewhere.

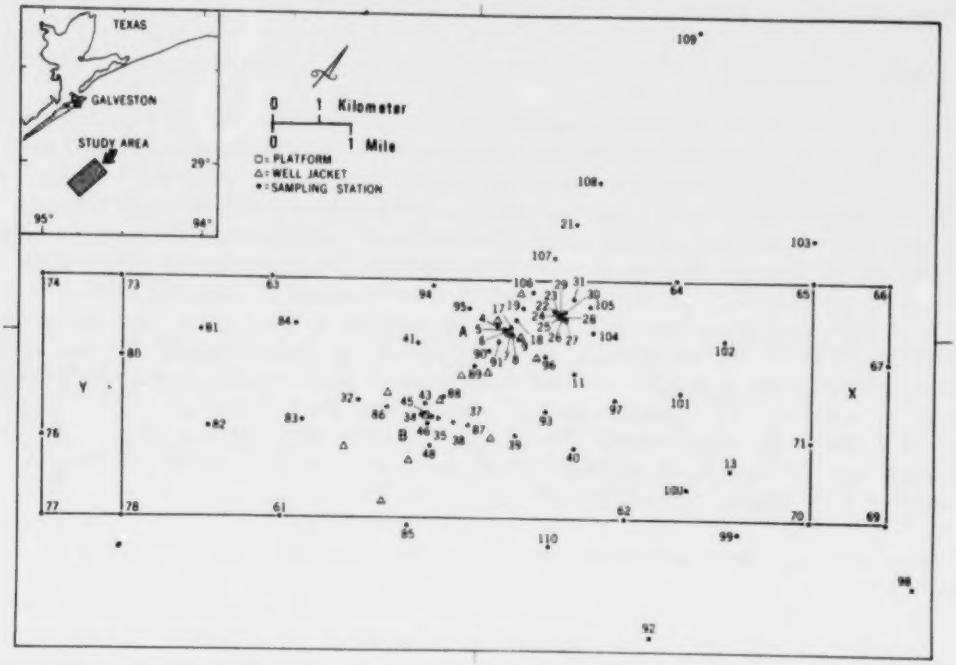

EXHIBIT 3: LOCATION OF BENTHIC STATIONS WITHIN THE BUCCANEER FIELD (These stations are used for collection of sediments and benthic fauna.)

Demersal Fish Collection

Trawl and trap collections of bottom fish were completed in May, August, and November 1976 and are scheduled for February 1977. To permit comparisons of species and sizes of fish and crustaceans, the sampling design includes collections in the two control areas and within the oil field complex. The presence of pipelines and difficulties of navigating a large vessel within the field made it impractical, however, to trawl close to structures, where species differences would be expected. Traps are only moderately effective in catching the species of interest. To date, 57 species of finfish and 23 species of crustaceans have been caught, but differences between areas are not apparent.

WATER COLUMN STUDIES

In this study area, we have analyzed the presence of ichthyoplankton, pelagic fish and structure-related fauna, and collected hydrographic data.

Ichthyoplankton

During the first year, plankton studies are limited to enumerating fish eggs and larvae in a further attempt to distinguish possible differences in fish inhabiting the control and oil field areas. Collections are on a quarterly schedule (May, August, November, and February), and data from the first two cruises have been processed. Oblique tows are made with paired Bongo nets (61 cm in diameter) that have mesh sizes of 0.333 mm and 0.505 mm. Each net is fitted with a flow meter to measure water volumes passing through the net. Neuston net tows are also made at each station to sample surface plankton. The mouth of the neuston net measures 1.0 x 0.5 m, and the mesh size is 0.505 mm. This net provides older larvae that are not vulnerable to the Bongo nets. Station locations for plankton collections include four within the Buccaneer Field, one in each of the two control areas, one situated 8 km shoreward of the field, and one an equal distance seaward. A total of 14,496 fish eggs and 2,439 larvae were caught during the May and August cruises. Among the larvae, 31 families, 33 genera, and 19 species were identified.

By far, anchovies were the most abundant group at both times. Total egg numbers were significantly larger in the field than at the control stations. Larval numbers were not clearly different in these areas, but more families were found in collections from the oil field.

Pelagic Fish

Many species of pelagic and reef fish are attracted to offshore structures; consequently, recreational fishermen and divers tend to concentrate their efforts near oil rigs. Observations of fishing activities on randomly selected days during the week and on weekends between June and early November indicated that 98% were operating within the oil field area. Of these, approximately 75% were bottom fishing, 20% were trolling, and 5% were diving. Red snapper constituted 86% of the bottom fishermen's catch, while king mackerel represented 58% of troller's catches. Divers appeared to divide their efforts among spadefish, red snapper, amberjack, and cobia. The number of fish speared per hour was considerably lower than the number taken by hook and line.

Attempts to use direct sampling methods to estimate stocks of the various pelagic species met with limited success. Each of the methods used (i.e., trolling, gillnetting, and longlining) is selective in terms of species and size of fish. Data developed from trolling in a standardized manner indicated that king mackerel are strongly attracted to platforms. Amberjack, cobia, and bluefish were caught only near structures. Surface and bottom gillnets as well as longlines caught fish but were damaged by sharks to such an extent that they were impractical to use. Currently, quantitative photographic techniques are being tried as a means of estimating species abundance near platforms.

Structure-related Fauna

Organisms attached to the supports of oil rigs represent communities that obviously would not be present in the absence of structures. Furthermore, they exist in areas of potential hydrocarbon pollution and may include useful indicator species. The composition and production rates of communities associated with the Buccaneer Field are being studied at Platform A and the southernmost satellite platform. Comparisons to an area without structures are made through counts of meroplankton and fish in a control area 8 km away from the field. Grazing rates by fish are inferred from stomach analyses. Information developed to date is primarily taxonomic but includes the observation that barnacles are numerous to depths of 10-14 m from the surface.

Effects of oil field structures on the migratory behavior of birds are being investigated through observations made from Platform A and from a vessel anchored in one of the control areas. Sightings during the spring migration period gave counts of 59 species near the platform and none in open water areas. A total of 61 birds that had died of exhaustion or starvation were found on the platform. None of these appeared to have collided with the structure. Only eight species were noted from the platform and five in the control area during the summer. Observations during the fall migration produced counts of 30 species from the platform, 10 in the control area, and no dead birds.

Hydrography

Hydrographic data are being collected to assist in development of diffusion models related to produced water discharges and trajectory models applicable to offshore oil spills. Routine temperature and salinity measurements are also made to support other investigations. Primary emphasis is placed on water flow characteristics as documented by current meter readings, drift bottles, and transponding buoys. Between May and October, 579 drift bottles were released in the oil field and control areas; of these 130 were recovered and returned. From July to October, 25 buoys were released to measure water movements at the surface and at 10 m below the surface. Positions of the buoys were fixed daily from shore stations over periods ranging from 1 to 15 days.

Transponding buoy data are extremely valuable as compared to data from drift bottles. Buoys permit analysis of short-term drifts resulting from wind or tidal influences and movements away from shore, whereas drift bottles are returned only if they reach the shore. To date, information from the two sources has indicated that shoreward drift components exist in many months when predominant flows are offshore or parallel to the beach.

POLLUTANT STUDIES

In this study area, we have analyzed hydrocarbons, trace metals, and total organic concentration.

Hydrocarbons

During the first year, objectives of this activity are to survey the hydrocarbon content of oil and discharges from production platforms and of water, sediments, and biota in the oil field and control areas. The biota include plankton, demersal and pelagic fish, benthos, if available, and barnacles from both production and satellite structures. Analyses are performed by gas chromatography and combined gas chromatography-mass spectrometry methods. Techniques are based on specifications of the Bureau of Land Management in its South Texas outer continental shelf study. Quality control samples have been analyzed by chemists at Texas A&M University and the National Analytical Laboratory, NMFS, Seattle, Washington. The high levels of hydrocarbons found in sediments near Platform A during the pilot study were absent during the summer of 1976. Comprehensive analyses and interpretations of study results are not available at this time.

Trace Metals

Investigations of trace metal concentrations include analyses of all bottom sediment samples, selected suspended sediments, barnacles, fish, and plankton. Determinations include barium, copper, chromium, cadmium, lead, manganese, mercury, strontium, and zinc. Currently available information suggests that lead and zinc concentrations in sediments near the two production platforms are well above background levels. Lead ranges from 5.5 to 11.8 ppm throughout the study area, except within 120 m of Platforms A and B, where values up to 100 ppm are common. Likewise, zinc concentrations range above 100 ppm in the vicinity of the two large structures, while the average value for the entire area is 41.5 ppm. Enrichment of chromium, copper, and cadmium sediments is not apparent. Analyses of other elements and other sample materials are not completed.

Total Organics

Ratios of C^{13}/C^{12} and C^{14} dating techniques are being employed to determine the depth and extent of sediments originating from drilling operations. Currently, samples obtained by long and short cores at stations radiating from the production platforms are being analyzed.

MODELING

Original study plans called for modeling studies to assist in integrating hydrographic and ecological data. These efforts were postponed until the first year of survey work had progressed to a point where requirements could be evaluated. In September 1976, a contract was awarded to develop a conceptual model and to outline second-year study plans that would produce the desired types of data. In October, this approach was discussed with members of a user group to elicit comments. The user group consists of representatives of eight state and federal agencies that may be expected to make use of information developed from the Buccaneer Field study. Their recommendations helped to ensure that ensuing studies will be responsive to needs of regulatory agencies.

The conceptual model, which summarizes trophic dependencies within the oil field ecosystem, will serve several functions during the second year of work. It is essentially a framework for managing activities and organizing information. As such, it should improve the efficiency of field activities and integration of results. When sufficient data become available, the conceptual model will be converted to a mathematical model to enable hypothesis testing and predictions of environmental impacts.

Research plans beyond 1977 will be designed to fill gaps in information required for the model and to verify impacts suggested by field observations.

DISCUSSION

Dr. Baumgartner: You said you could not find any evidence of drilling muds?

Dr. McRae: There is a definite pattern in the organics, as along the coast, that indicates that the older material is there, but so far, it is not correlated to the currents or this work.

Dr. Baumgartner: But you did say you found some on the basis of zinc and lead near the platforms. How close?

Dr. McRae: As close as the batteries thrown over the side. However, we are working under the platform with the divers, and the pollution can be carried by a current. Next year, we will examine some of the fish that have sores. Quite a few of these reef-type fish that are associated with the platforms have sores, which may be carcinogenic.

From the floor: Do the fishermen, the recreational fishermen, remark upon the quality of the fish that they catch?

Dr. McRae: They notice the sores; but they can cut them out with a knife, so it doesn't bother them.

A STANDARD PROGRAM FOR ENVIRONMENTAL IMPACT ASSESSMENT: PHASE I—ICHTHYOPLANKTON SAMPLING

Martin A.M. Hyman, Ernesto Lorda, and Saul B. Saila*

The pressing need for enlightened management and regulation of estuarine ecosystems is made more critical by the difficulty of predicting the consequences of man-induced perturbations. An estuary is a very complex system with large numbers of biological, physical, and chemical components. The physical variables vary with time at a fixed point in space, mainly as a result of water movement of various scales according to the principles of dynamic oceanography. Biological variables and some chemical variables vary as a result of the same processes, but also according to principles inherent in biological material. These may be independent of physical water movements or may enhance or reduce their effects. Interactions among these components are characteristically nonlinear and involve intricate feedback loops.

An adequate or comprehensive survey of attempts to model ecosystems or even specific estuarine ecosystems involves much more effort than is available for the purpose at hand. Suffice it to say that most predictive modeling efforts to date have involved viewing ecosystems as consisting of interconnected compartments (sometimes of several component species), and interest is centered on the amount of energy or material in each compartment and the flow rates between compartments. The ecological processes going on within the compartment among various species are assumed to average out into some fairly simple behavior for the compartment.

The complexity of estuarine ecosystems and the growing need for proper management decisions and regulations have placed a high priority on large-scale integrated models. One example of a complex simulation model for Narragansett Bay is the recent work by Nixon and Kremer (1975). The practical effectiveness of predictions from a model, such as the above-mentioned, is limited by the size of the model grid as well as by the accuracy and precision of the model parameters and state variables, among other things. In general, it appears that ecosystem level models, as currently conceived, are able to predict with reasonable fidelity only the consequences of very gross types of perturbations and cannot be used for specific practical decisions.

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Recognizing the limitations of these ecosystem models, environmental impact studies have historically developed a so-called baseline and a so-called monitoring program. As currently executed, baseline studies usually consist of a sort of "snapshot," a short-term picture of an environment, which is variable in both space and time and with little indication of the magnitude of these scales of variability. The monitoring program has evolved into a systematic collection of data over time. Unfortunately, little critical information on the sampling interval, the minimum desired length of the time series record, or the nature of sampling variability is available.

Among the host of economic, political, logistic, and statistical problems involved in the establishment of a standard program for environmental impact assessment, the latter two areas have received insufficient attention, in spite of their potential value. Relatively little has yet been done to utilize the principles of experimental design in sorting out effects of spatial and temporal variability as they affect responses of organisms.

In general, it is apparent that the intended use of a particular data set will determine a particular sampling program, and no arbitrarily drawn system for sampling will be suitable for monitoring. The reasons for this include the fact that a sampling program for a particular experiment involves several factors, among which the sampling rate, the duration of individual observations, the accuracy and precision of observations, as well as the accuracy and precision of timing (synchronization) are important.

It is suggested that a successful environment impact assessment program is predicated on a sequence of carefully planned experiments designed to answer specific questions, rather than on a general measurement system. This carefully planned standard program can then be used for establishing suitable guidelines for more routine types of assessment.

To assign sources of variability in ichthyoplankton surveys more accurately, an intensive sampling scheme was proposed for a single site. The purpose of this study was to work toward producing a standard program for ichthyoplankton sampling. If variability is assigned to various effects, as opposed to being confined into an overall error term, data will be of an overall higher consistency and, thus, generally more useful. Specifically, once this variability is better understood, any modeling effort should prove more productive.

COLLECTION OF DATA

To intensively look at variability within a station, we chose a single sample site in West Passage of Narragansett Bay. Because this was an "average" site, it was felt that its results will be directly applicable to any sampling program. While interstation variability is important and assumed to exist, it was not within the scope of our study.

The selected sampling site was 1/4 mile off the southwest tip of Prudence Island. This area has a relatively uniform depth of approximately 50 ft and is not located in a high traffic zone. The 50 ft depth permitted 4 min duration double oblique tows to be taken. The chosen site was marked by bearings and fathometer traces and buoyed to ensure geographic repeatability.

Beginning at 8 a.m. for 24 hours on 24 June, 28 June, 1 July, and 7 July 1976, sampling was conducted hourly for 24 hours. Each hour, two tows were taken, one with the tide and the other against it. A total of 335 samples was taken. Although problems were encountered on day 1, virtually complete sample sets were taken on each of the subsequent three days. These last three days comprise our data set. Consequently, for the purpose of analysis, only 280 samples were taken. The initial design called for sampling over a 2-week period; however, the program did run over into a third week.

The sampling gear chosen for the study was 60 cm Bongo nets, both sides containing 505μ mesh. Because of the large mass of historical data on this gear, it seemed an appropriate starting point in spite of recent additions to the gear collection. It was expected that the two-net system would produce replicate tows; however, A-B variability was measured. Two General Oceanics flow meters were used: one mounted inside one of the nets and the other external to the pair. For all practical purposes, this meter is the existing industry standard. In addition, a dual-axis electromagnetic current meter with deck readout was incorporated into the gear.

In the original sampling design, we proposed to compare the results obtained from an electromagnetic (EM) current meter and General Oceanics (GO) flow meters. It was initially felt that the GO meters were a questionable source of volume information. However, the meter comparison proved impractical. Although several successful tows were made with the EM internal to the nets, when it was placed externally, an irreparable loss of cable continuity resulted in premature termination of the experiment. This loss was not correctable within our sampling period; however, the nets fished positively throughout the initial tows and did not backwash at any time.

As a result of the EM failure, the GO meters became the sole source of volume data. Only volume data and average speed were obtainable from these units; however, the meters did prove to be remarkably consistent. Three meters were used and rotated each 8 hours to check for meter bias. None was determined to exist. For each tow, internal and external readings were taken. In all but several cases, the internal meter reading was between 10% and 17% higher than the external meter. This information is consistent with the findings of the MARMAP studies (personal communication with Robert Marak, National Marine Fisheries Service). The consistency of the ratios of internal to external readings deviated by less than 5% over 168 tows. We considered this consistency to be more than acceptable for the purposes of our comparisons.

In conjunction with the sampling program, various auxiliary data were collected. Ambient light was measured by a standardized light meter reading taken in a northerly direction 30° over the horizon. Cloud cover and wind speed were estimated in a standard manner. A water temperature profile was taken hourly. No salinity data were collected.

The platform used for sampling was the University of Rhode Island's R.V. Dulcinea, a 39 ft converted U.S. Navy utility boat. Engine rpm were maintained constant to eliminate differential organism response, except as it related to tidal and wind effects. Gear was lowered to just above the bottom with the vessel at standstill. When an appropriate length of two wires was let out, the tow began. Attempts were made to maintain average vessel speed at 2.5 kt (1.25 m/sec). This technique varies from traditional sampling with Bongo nets in that the vessel is stationary when the gear is deployed; thus, "fishing" does not begin until desired and the meter readings reflect the tow period more accurately. Checks made with the electromagnetic meter showed that this technique produced a uniform tow velocity. Based on the MARMAP program of the National Marine Fisheries Service, a recovery rate of 0.33 m/sec was used, which resulted in an average net velocity of 1.6 m/sec.

All the samples were transported to Marine Research, Inc., for identification and counting. To determine subsampling error, 10% of the samples were counted entirely: one-third of these were from sparse samples, one-third from intermediate samples, and one-third from dense samples. For purposes of this check, Marine Research, Inc., personnel estimated density, and 10% of the samples were recounted to evaluate operator error. Finally, in addition to organism counts, biomass estimates were computed for each sample using volume displacement. The samples were sealed in preservative for purposes of archiving.

The samples were analyzed for ichthyoplankton eggs and larvae and zooplankton and benthic organisms. Because of its mesh size and design, the gear was not efficient for zooplankton or benthics; however, we felt that this lack of efficiency was uniform over the sampling period. Thus, it would give some indication of variability in a relative sense.

The data collected were separated into two categories: physical data and species counts. The species counts were further separated into four classes: ichthyoplankton eggs, ichthyoplankton larvae, and zooplankton and benthic organisms.

For each sample, the number, hour, tow direction, wind speed, surface temperature, mean water column temperature (weighted), bottom temperature, water level function, light intensity, tidal factor, inside volume, outside volume, time of first oblique tow, time between tows, and time of second oblique tow were recorded. With the exception of the water level function and tidal factor, each parameter is self-explanatory. The water level function and tidal factor were computed from the times and heights for the high and low tides for those days. Times and heights were corrected for Prudence Island (Exhibit 1).

The water level function is defined as

$$WL = f(t) = am \cdot [(\sin t) + 1] + min,$$

where

WL = water level

t = point in time expressed in radians or degrees

am = 0.5 (tidal amplitude)

min = WL at low tide (minimum water level)

max = WL at high tide (maximum water level);

so that

$$am = \frac{max - min}{2}.$$

The tidal factor is formally defined as the instantaneous rate of change of the water level in meters per unit time. It is the first derivative of the above function, so that:

$$TF = f'(t) = \frac{dWL}{dt} = am \cdot \cos t,$$

where

TF = tidal factor (or slope of the water level function WL)

Negative values correspond to ebb tide or southward flow

Positive values correspond to flood tide or northward flow

Zero corresponds to slack tide.

ANALYSIS OF DATA

There are two basic sources of variability in plankton data: that which occurs naturally and that which is introduced in the analysis. We assumed that the samples, if collected rigorously, are subject to as little human-induced variability as possible. In the counting and identification stage, any variability is human introduced.

To examine relationships among species counts, we will complete correlation matrices. Then, the species groups that demonstrate the greatest affinity in response will be correlated separately with the physical parameters and tow speeds. The results of this two-step correlation analysis will be used in determining the actual sources of most of the observed variability in the species counts. Multivariate analyses of variance will then be conducted to investigate the response of individual species and/or major species groups to the most relevant variables.

The final model used in the above analysis cannot be established *a priori*, since its construction will be based on the results of the correlation analysis. However, it is expected that the response of several major species (dependent variables) will be measured for roughly the following sources of variance (independent variables):

Main Effects	Levels	D.F.
D = direction of tow	2	1
H = time of day (within day variance)	24	23
M = days (between day variance)	3	2
R = replicate tows (2 nets each tow)	2	1

The effect "time of day" will be characterized by physical factors, such as water temperature, light, and tidal factor, on the basis of the correlation analysis. Any nonsignificant variable will be pooled into the error term.

To estimate the variability introduced in the counting and identification stages, the sample counts will be examined for counting error. For each of the whole counts, comparisons with the intermediate split counts will be made. In addition, the recounts will be compared to the initial counts.

DISCUSSION

From the floor: Did you do your tows both in daylight and at night?

Dr. Hyman: Yes, we started at 8:00 a.m. and ended at 7:00 a.m. the following morning -- 24 hrs.

From the floor: How much tide flow did you have in there?

Dr. Hyman: Oh, a foot and a half.

From the floor: You have more than a foot and a half, don't you?

From the floor: Neap tide, 2½; spring tide, maybe up to 4 ft.

EFFECTS OF CHEMICAL USE IN OFFSHORE WELL-DRILLING OPERATIONS

Norman L. Richards*

Recent environmental impact statements on oil and gas lease sales in the Gulf of Mexico have devoted proportionately little discussion to the environmental aspects of chemical use in well-drilling operations. This may reflect the fact that few studies have been done, and most are acute static bioassays that have little relevance to natural conditions such as drilling mud discharges (Sheen Technical Subcommittee, 1976). Of those acute bioassays in the literature, few have reported adverse effects of drilling fluids or their components. Field observations have revealed a variety of marine life in the vicinity of drilling rigs (Fisher, 1975). In addition, it is generally assumed that fluids would either have only a limited local effect near the plume discharge point or would rapidly be dispersed and diluted in the field (Sheen Technical Subcommittee, 1976).

Research on the effects of drilling muds is further complicated by the chemical complexity of muds. Additives contain: pH control products, bactericides, calcium removers, corrosion inhibitors, deformers, emulsifiers, filtrate reducers, flocculants, foaming agents, lost circulation materials, lubricants, shale-control inhibitors, surface-active agents, thinners, dispersants, viscosifiers, and weighting agents (Wright, 1975). Analyses of potential effects of chemicals used in well-drilling operations must take into account the variation in mud and cutting composition from the type of substrate drilled, well depth, availability of mud components, temperatures generated, relative cost of components, operator experience, etc. (Fisher, 1975).

It is logical that the "King-Muir" report included the following gas and oil environmental effects objective: "Objective A: Determine the fate and effects in marine and estuarine ecosystems of pollutants of oil and gas extraction activities...", and projected as a national need: "...Project 2: Determine the ecological effects of marine and estuarine organisms of pollutants from oil and gas extraction." (Working Group on Health and Environmental Effects of Energy Use, 1974). A rigorous biological assessment of potential environmental effects of

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chemicals used in well-drilling operations has many technical difficulties and is extremely resource intensive. Because of these considerations, a limited program to study the effects of drilling mud components was undertaken at the EPA Environmental Research Laboratory at Gulf Breeze, Florida. Exhibit 1 illustrates some areas in which an understanding of the effects of drilling muds is lacking, i.e.,

- Estimates of the distance that rigs must be from coral reefs to minimize effects from emissions
- Effects of mud on resident rig marine life
- Advisability of aquaculture near platforms
- Recommended distance from rigs for seafood harvesting.

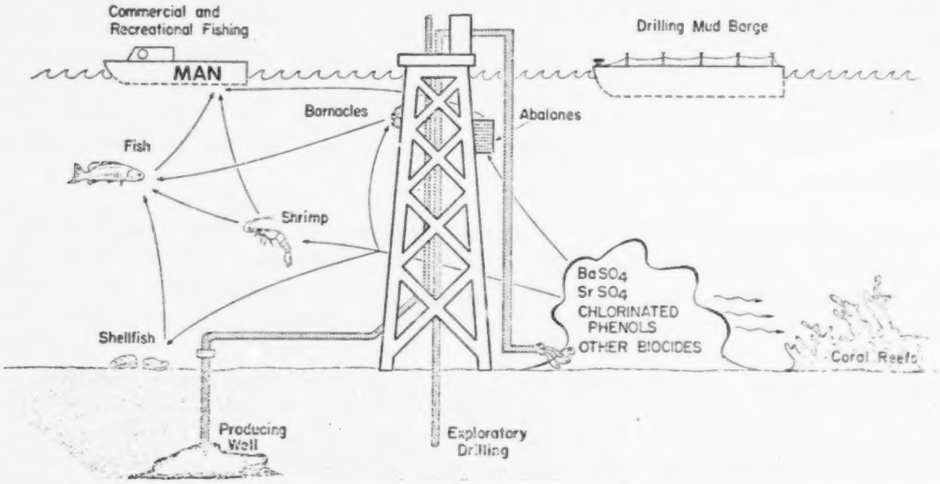

EXHIBIT 1: SCHEMATIC DIAGRAM OF THE POSSIBLE FATE OF ADDITIVES

METHODS

Our approach to an assessment of the effects of chemicals used in well-drilling operations is shown in Exhibit 2. The experimental design adheres to a tiered screening sequence, which has progressively more complex and time-consuming tests to be run on progressively fewer compounds. Xenobiotics are selected from lists of drilling mud components in current environmental impact statements, with emphasis on those characterized by heavy usage patterns or having suspect toxicity.

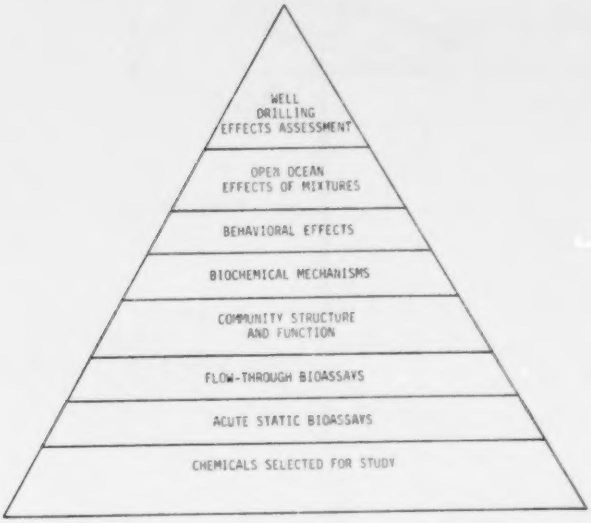
WELL DRILLING EFFECTS ASSESSMENT
OPEN OCEAN EFFECTS OF MIXTURES
BEHAVIORAL EFFECTS
BIOCHEMICAL MECHANISMS
COMMUNITY STRUCTURE AND FUNCTION
FLOW-THROUGH BIOASSAYS
ACUTE STATIC BIOASSAYS
CHEMICALS SELECTED FOR STUDY

EXHIBIT 2: ASSESSMENT OF THE EFFECTS OF CHEMICALS USED IN WELL-DRILLING

Preliminary screening of selected compounds is accomplished by acute static 96 hr bioassays. They are conducted with drilling fluid constituents by using laboratory-reared sheepshead minnows (Cyprindon variegatus) and grass shrimp (Palaemonetes pugio). These range-finding tests are helpful in selecting toxicant concentrations for subsequent flowing sea water bioassays for each compound, component, or mixture of interest.

Flow-through toxicity methods were selected for a second tier of testing because they more nearly approximate in situ conditions. In contrast to static tests, metabolic products and excreta are removed, while oxygenated sea water and toxicant are continuously supplied. Data obtained from flow-through toxicity tests are generally preferable over static tests as the more precise measure of toxicity and bioaccumulation. A few compounds are selected from flow-through experiments for the next tier of testing: the composition and functions of estuarine communities.

The effect of selected xenobiotics on colonization of planktonic larvae and microorganisms is analyzed by means of the apparatus developed at the Gulf Breeze Laboratory by Hansen (1974). Exhibit 3 illustrates the apparatus used in these studies. Sea water, with its natural component of plankton and microorganisms, is pumped from the estuary adjacent to the laboratory into the primary constant head box. Xenobiotics are continuously metered into water after they are siphoned from the primary to the secondary constant head box; the control apparatus receives the same flow of water. Water then flows from the secondary constant head box to each of the 10 adjacent aquaria, i.e., 10 replicates for each treatment, including controls. At the end of a 9-week exposure

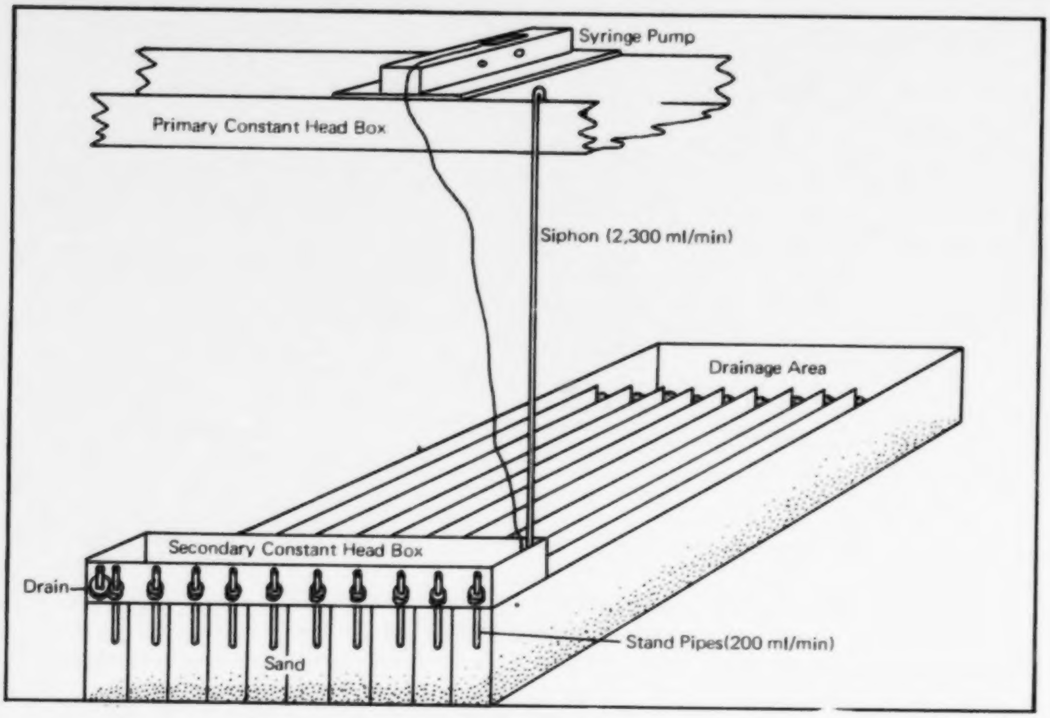

EXHIBIT 3: APPARATUS USED TO TEST THE EFFECT OF XENOBIOTICS ON COMPOSITION OF ESTUARINE COMMUNITIES

period, microflora, macrofauna, and meiofauna are sampled. To determine the effect of the xenobiotic after each treatment, numbers and species of microflora, meiofauna, and macrofauna are compared in control and exposed aquaria, and concentrations of xenobiotics in test water and sediment are determined. Samples of water from the constant head boxes are taken twice a week, and sediment cores from aquaria are taken from each apparatus at the end of the exposure.

RESULTS

Two compounds were selected for further study from those screened in acute bioassays. Barium sulfate was chosen because "...barite discharged with drilling muds is a nontoxic substance," and pentachlorophenol was chosen because it is a "...potentially hazardous" drilling mud constituent (Sheen Technical Subcommittee, 1976).

Static toxicity tests with sodium pentachlorophenate have been conducted with larval stages of three marine/estuarine species. The species, the LC_{50} values obtained, and the respective exposure period were: eastern oyster, < 100 ppb, 48 hr; grass shrimp, 649 ppb, 96 hr; and pinfish, 38 ppb, 96 hr. A similar study with Dowicide G (79% PCP) and pinfish larvae resulted in a 96 hr LC_{50} of 66 ppb. A bioconcentration study with oysters exposed to sodium pentachlorophenate in flowing sea water for 28 days demonstrated the bioconcentration of 80 times that measured in the exposure water.

Effects of pentachlorophenol on development of estaurine settling communities were determined by using the apparatus devised by Hansen (1974) and described under the methods section. The macrofauna results from a 9-week exposure are shown in Exhibit 4. Individuals and species of the numerically dominant phyla (Annelida, Arthropoda, and Mollusca) decreased as concentration increased. Sensitivity differed among species of the same phylum. For example, the population of the annelid Haploscoloplos was reduced by exposure to 96 μg/ℓ PCP, but not Capitella capitata. Molluscs markedly decreased at 7 μg/ℓ; annelids and arthropods at 76 μg/ℓ. Few macrofauna occurred at 622 μg/ℓ. Total number of animals and species were significantly less ($\alpha = 0.01$) in aquaria exposed to 76 μg/ℓ than in those unexposed or exposed to 7 μg/ℓ. Therefore, pentachlorophenol altered the structure of experimental macrofauna communities that developed during exposure by changing the relative abundance of animals by species and phylum.

Meiofauna community structures were studied in the same tanks as the macrofauna. Exhibit 5 summarizes data on the basis of rarefaction. Although there are structural changes in the community, this is not demonstrated by any of the species diversity indices shown: "H" designates Shannon-Weaver Composite index; "J," equibility; and "SR," species richness.

Exhibit 6 shows the shift in nematode feeding types as a function of pentachlorophenol concentration. An analysis of the species revealed that the meiofauna population shifted to predominantly detritis feeders in 622 μg/ℓ of pentachlorophenol. The symbols used are: 1A, selective deposit feeders; 1B, nonselective deposit feeders; 2A, epigrowth feeders; and, 2B, omnivores. Therefore, the functional types of meiofauna shift as a fraction of PCP concentration.

This experiment indicates that the test system described with meta oans may have distinct advantages; they have a relatively short life cycle; adults normally will reproduce continually; they invade the system rapidly. Therefore, structure and functions of communities can be determined.

Exhibit 7 summarizes the aerobic microbial colony forming units observed in the presence and absence of pentachlorphenol. The number of cultivatable microorganisms was significantly lowered in aquaria with high pentachlorophenol concentrations.

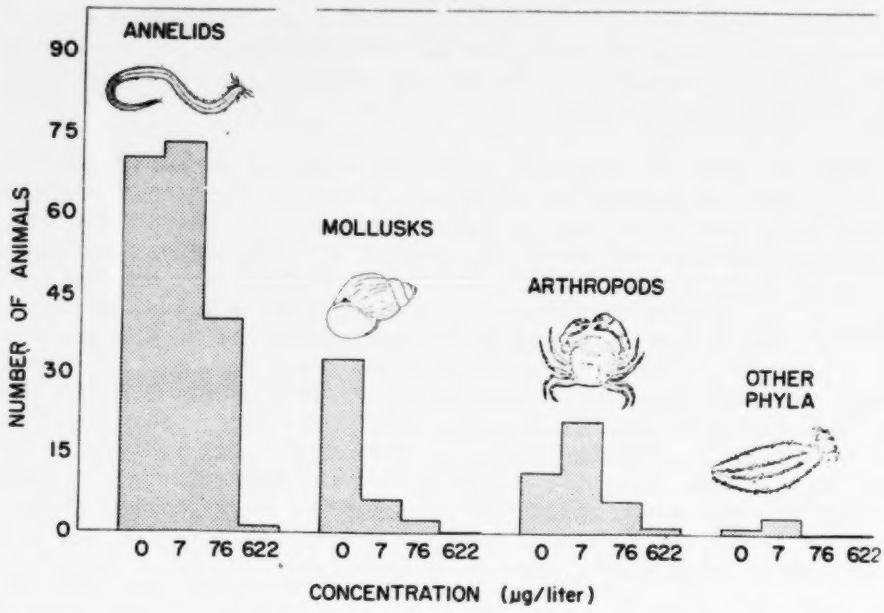

EXHIBIT 4: SUMMARY OF 6-WEEK MACROFAUNA COMMUNITY DIVERSITY STUDY WITH PENTACHLOROPHENOL

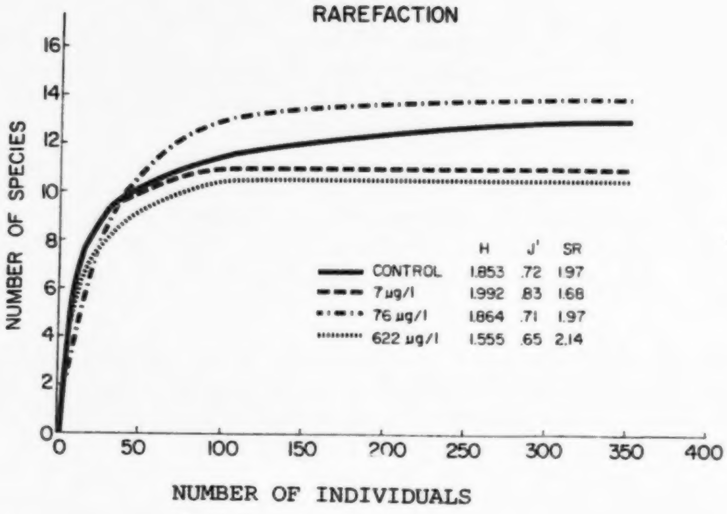

EXHIBIT 5: SPECIES DIVERSITY INDEX OF NEMATODES EXPOSED TO VARIOUS CONCENTRATIONS OF PCP

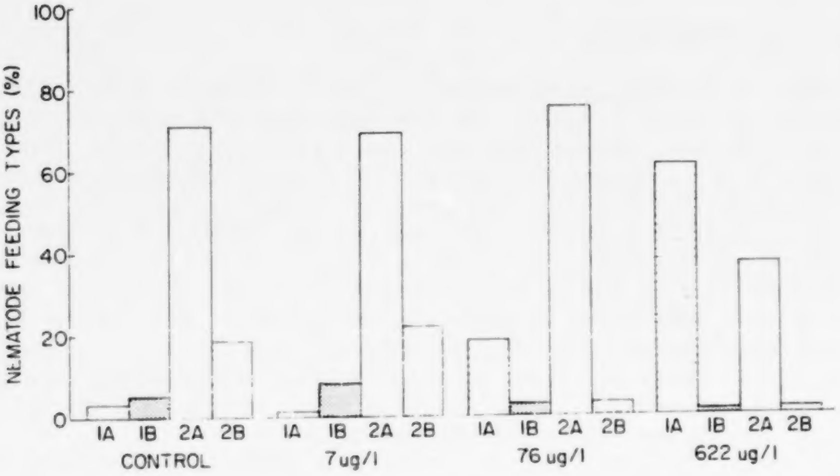

EXHIBIT 6: EFFECT OF PCP ON
MEIOFAUNA COMMUNITIES

Microbial Colony Forming Units

	Control	500 ppb Pentachlorophenol
$\bar{x}$	15.93×10^5	6.18×10^5
σ	10.75	4.81

$t_{exp} = 2.85$

$t_{exp} = 2.26$ (95% confidence)

EXHIBIT 7: EFFECTS OF PENTACHLOROPHENOL
ON MICROBIAL POPULATIONS

Exhibit 8 is a picture of control grass shrimp (Palaemonetes pugio) that have not been exposed to barite. Exhibit 9 shows barite-exposed shrimp with the intestinal tract (white) filled with barite: the shrimp ingested the material.

SUMMARY AND DISCUSSION

Data on the effects of chemical use in offshore well-drilling operations are very limited. Policy decisions are currently based on static, 96-hr LC_{50} determinations, observations of divers, and theoretical models of pollutant dispersion. A limited research program on drilling-fluid constituents has been initiated at the Gulf Breeze Laboratory to provide a better data base as one component in the evaluation of the relative hazard of using alternative drilling mud constituents and to develop better laboratory methods for xenobiotic evaluation. It is obvious that these methods should include: effects on the structure and function of communities, indirect effects of pollutants, bioaccumulation potential, toxicity of mixtures to organisms indigenous to lease areas, attraction of marine species to chemicals used in well-drilling operations, and mechanism of action of toxicants. In this way, choices among the multitude of alternative chemicals available for well-drilling operations can be based on better toxicological information. Hard data on effects might also eliminate unjustified concern about effects of certain chemicals, which, in fact, may be safely used in well-drilling operations.

Exhibit 10 itemizes the study elements and the principal investigators for the first half year of research on chemical use in offshore well-drilling activities.

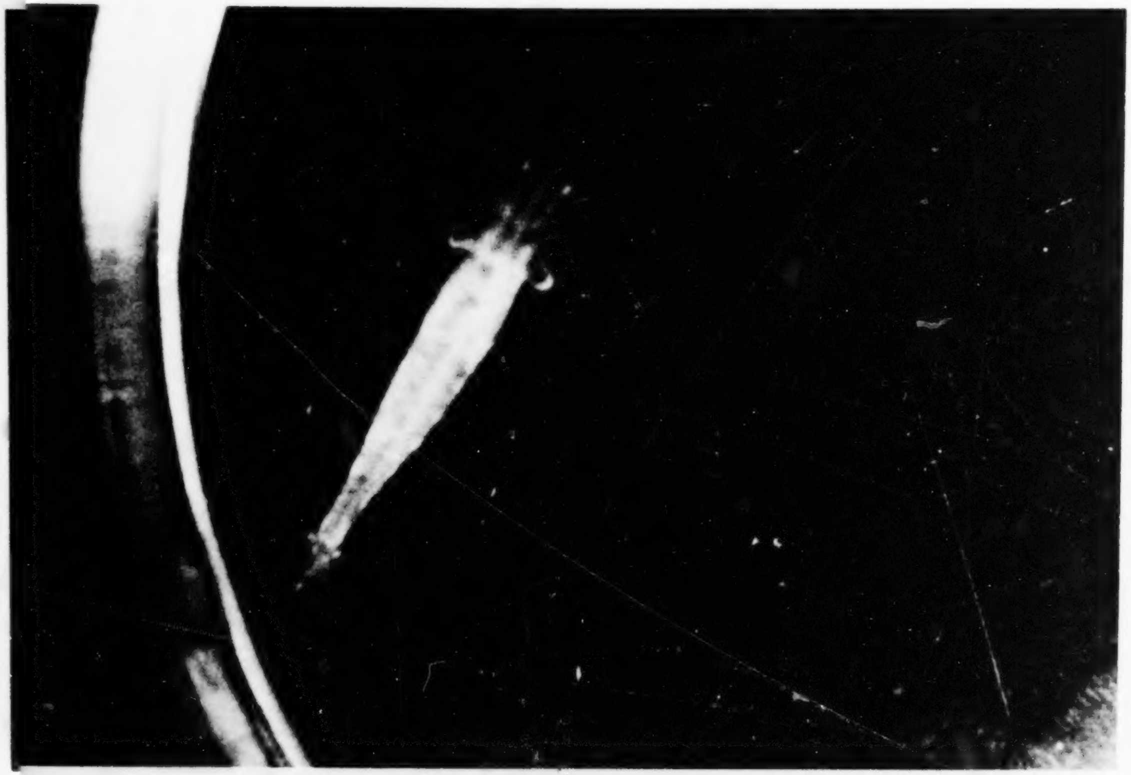

EXHIBIT 8: CONTROL GRASS SHRIMP (PALAEMONETES PUGIO)

EXHIBIT 9: GRASS SHRIMP (*PALAEMONETES PUGIO*) EXPOSED TO BARIUM SULFATE

EXHIBIT 10. LIST OF STUDY ELEMENTS, PRINCIPAL INVESTIGATORS, AND GRANTEES DURING THE FIRST HALF YEAR OF RESEARCH ON CHEMICAL USE IN OFFSHORE WELL-DRILLING ACTIVITIES

Study Element	Investigator	Affiliation
Planning Workshop	M. Summers	University of Texas
Chemical Analysis	J. Moore	EPA, Gulf Breeze
Chemical Analysis	A. Wilson	EPA, Gulf Breeze
Statistical Analysis	J. Ogelsby	University of West Florida
Atomic Adsorption Spectroscopy	A. Brennan	University of West Florida
Static Acute Toxicity	P. Borthwick	EPA, Gulf Breeze
Static Acute Toxicity	S. Schimmel	EPA, Gulf Breeze
Static Acute Toxicity	J. Patrick	EPA, Gulf Breeze
Flowthrough Toxicity	S. Schimmel	EPA, Gulf Breeze
Flowthrough Toxicity	J. Patrick	EPA, Gulf Breeze
Macrofauna	M.E. Tagatz	EPA, Gulf Breeze
Macrofauna	J. Ivey	EPA, Gulf Breeze
Macrofauna	N. Tobia	EPA, Gulf Breeze
Meiofauna	F. Cantalmo	University of West Florida
Microbiology	E. Suarez	University of West Florida
Biochemistry	F. Fox	University of West Florida
Physiology	R. Rao	University of West Florida
Physiology	A. Cantalmo	University of West Florida
Pathobiology	-	-
Photography and Illustrations	S. Foss	EPA, Gulf Breeze

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DISCUSSION

Dr. Baumgartner: You stated that drilling muds are pumped overboard and released at the base of the platform. Is that the general practice or is some of this recycled?

Dr. Richards: You have to make a distinction here between the drilling operation and production. During the drilling phase, some of these materials are recycled. Some of the Gulf of Mexico environmental impact statements summarize the tonnage data on the amounts that actually go overboard.

Dr. Baumgartner: Is it fair to ask how many tons per day are discharged?

From the floor: It depends on the tonnage of the well.

Dr. Richards: A table in Lease 47 Environmental Impact Statement gives that.

From the floor: Do you know how much biocide is used to cut the rust impact on the structures?

Dr. Richards: At the Houston conference, held by EPA about a year ago, the types and quantities of biocides used were summarized. Biocides are occasionally used to inhibit microbiotic growth, which causes corrosion. This poses a particular problem for the EPA because of discharge permits from the platforms. I do not have the data on the quantities used to minimize the bacterial growth in the gas production, but there are figures on the other uses. One other point I might also make is that barium sulphate is used as a weighting agent, and the barium sulphate helps the growth of several bacteria, which in turn, produce corrosive end-products. To keep down the bacteria, the biocides must be added.

Dr. McCammon: Are you planning to look at any other metals, such as chromium?

Dr. Richards: As a matter of fact, I have only discussed a small amount of the things that we have already screened. I wanted to give one example of something that was supposed to be totally negative and one that I thought was positive. But, in fact, we have screened chromium sulphanate, and we will be going into the community studies with that compound shortly. We are interested in that particular compound because it has a heavy usage pattern in the Gulf of Mexico. In addition, I have heard from one of the drilling mud salesmen that The Netherlands has put some restrictions on its usage in the North Sea.

Dr. McCammon: Are you going to try and define the chemical form of the metals, because I hear that it is hexavalent chromium that is coming out?

Dr. Richards: As you noticed in that pyramid, we had mechanisms at the top. We are attempting to define the mode of action. If we can understand the mechanism, then we can extrapolate to different organisms and to different situations.

From the floor: I do not believe that EPA exerts any control over drilling rights. I think we only have jurisdiction over oil. The General Council has not ruled on whether or not we have anything to say per se in terms of MBES drilling permits.

THE CHEMISTRY AND ECOLOGICAL EFFECTS OF CHLORINATION OF SEAWATER: A SUMMARY OF EPA RESEARCH PROJECTS

W.P. Davis,* D.P. Middaugh,* J.H. Carpenter,†
G.R. Helz,** and M.H. Roberts, Jr.††

The use of chlorination to disinfect for bacterial control or control biological fouling is a widespread process unique to the United States. Many countries, including Sweden, Norway, West Germany, Great Britain, and France, have been very conservative in the use of chlorine for these applications. Recent surveys have linked fish mortalities and possibly shellfish bed recoveries to excessive chlorination or reduction of chlorination, respectively (Virginia State Water Control Board, 1974; Virginia Institute of Marine Science, 1975). Studies by Jolley (1973), Helz (in manuscript), and Carpenter (1976, 1977) reveal that both halogenated and nonhalogenated compounds are produced from chlorination of waters rich in organic compounds from natural productivity.

In this paper, we summarize the results of ongoing research projects from 1975. In it, we relate preliminary findings to needs for further investigation of the effects of chlorination on marine ecosystems.

The annual production of chlorine in the United States was estimated as 95 x 10^{11} kilos (10.5 million tons) in 1975 (White, 1976). The chemical industry uses 80 percent; the pulp and paper industry utilizes 16 percent; 3 to 4 percent is used for disinfection, including municipal treatment, household uses, antifoulant in cooling waters, swimming, and potable water treatment. A recent estimate states that 200,000 tons of chlorine are used in disinfection. Among the 50 states

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surveyed none plan any reduction in these chlorination practices. No statistical data seem available to estimate the proportions of usage directly or indirectly affecting marine waters. It is reasonable to state that the high "chlorine demand" of coastal waters results in high utilization wherever these waters are used as cooling for power plants. Municipal treatment plants also contribute a substantial, if not greater, level of chlorination to estuarine and coastal waters.

Since 1975, the Gulf Breeze Environmental Research Laboratory, Bears Bluff Field Station, near Charleston, South Carolina, has been coordinating a study of the marine aspects of chlorination. This effort, with extramural grants supported through Environmental Protection Agency's (EPA) Office of Energy Minerals and Industry (OEMI), is designed to establish a scientific basis for decisions guiding future energy production and usage patterns, and environmental protection measures.

CHEMISTRY OF CHLORINATION OF MARINE WATERS

Marine waters are characterized and defined by the levels of dissolved halides, principally chloride and bromide. Unlike the situation in fresh water, strong oxidants added to marine waters react nearly instantaneously with natural bromide and produce oxidative states of bromine, including hypobromous acid, hypobromite ion, and bromate (Carpenter and Macalady, 1976; Carpenter *et al*., 1977). These findings are significant because the most popular method currently used to estimate "residual chlorine" does not provide accurate determinations of hypobromite; therefore, the input to the environment is seriously underestimated (Carpenter *et al*., in manuscript). In addition, this amperometric titration method does not sense bromate at all; yet this form of bromine may persist in marine environments for long periods of time with unknown ecological effects (Carpenter *et al*., 1977).

Additionally, chlorination of sea water produces reaction products from halogenation of both natural and man-produced organic compounds. Natural sea water chlorinated in the laboratory produces chloro-bromo analogs not found in unchlorinated sea water (Carpenter, 1977; Carpenter and Macalady, 1976).

Analyses and examination of these reaction products required modification and development of demanding techniques. Four major steps are involved:

1. Isolation and concentration of the organic fraction from the bulk aqueous phase

2. Separation of the organic fraction into individual compounds

3. Detection and characterization of each compound

4. Final identification of each compound.

Separation and characterization of compounds trapped from the isolation and concentration step are performed by a combination of gas chromatography and mass spectrometry methods. Since as many as 150 compounds may be separated in a single run, a minicomputer system is used to accumulate the copious data produced. Organic compounds are identified by reference to laboratory standards and to computer libraries, where the mass spectra may be compared to the over 41,100 compounds on file.

These investigations, conducted at the School of Marine and Atmospheric Sciences, University of Miami under Dr. James H. Carpenter, have isolated a large number of compounds and tentatively identified 20 of these. Chlorination produced bromoform, bromoxylene, bromomesitylene, dibromochlomethane, and chloroform. Many other compounds are obviously present and are being investigated using high-resolution capillary columns. Chlorination of sea water exposed to sunlight increases formation of halogenated organics. In the case of bromoform, sunlight increases production approximately tenfold, yielding concentrations of several hundred ppb (Carpenter, personal communication).

Analyses and inventory of these by-products are continuing. Formation of halogenated compounds with low solubility is being examined with an eye to the entry of these forms into marine food webs, as previously known for many pesticides.

Investigation into the detection of compounds from chlorination in the cooling water effluent waters from a power plant is one of the principal emphases of a research grant to the University of Maryland, under the direction of Dr. George R. Helz. These investigations have detected bromoform in the chlorinated cooling effluent waters. Furthermore, it has been demonstrated that chlorination of waters with natural "humic acids" can produce nitriles such as hydrogen cyanide (Hsu and Helz, in manuscript). Further work must be performed to better define the production rates of both these compounds.

The methodology for testing potential biological uptake and accumulation is being modified from work on pesticides. Still, bromoform and cyanide pose special problems since little experience exists for tissue analysis in aquatic organisms for these compounds.

ECOLOGICAL IMPACT OF CHLORINATION ON MARINE ORGANISMS

Much biological work is yet to come as the chemical characterization and identification tasks continue. Initial phases of biological investigations involved compilation and review of available data on toxicity of chlorination (Davis and Middaugh, 1976; Middaugh and Davis, 1976). Published results were typically expressed as the toxicity of so-called "total residual chlorine," which as we previously mentioned (Carpenter et al., in manuscript), is technically and scientifically incorrect for marine waters. It is immediately apparent that far less information exists for biological effects of marine water chlorination compared to fresh water situations (Brungs, 1973; Davis and Middaugh, 1976; Jolley, 1976).

To achieve close communication of ongoing research projects, a workshop was held at Chesapeake Biological Laboratory. Here, investigators agreed that "chlorination-induced oxidants" should be the proper terminology used for active oxidants affecting the readings achieved on amperometric titrators. Additionally, 15 papers were presented that summarized the state of the art for inorganic and organic chemistry, biological toxicology, and physiology of marine waters (Chesapeake Science, 18).

Toxicological studies have been carried out both extramurally at the Virginia Institute of Marine Science on molluscs and decapod crustaceans and with fishes at Bears Bluff Field Station. Investigations on decapod crustaceans have established toxicity data for larval crabs and for the bivalve mollusc Mulinia lateralis. Dr. Morris Roberts has also directed investigations on the seriological effects on the blue crab (Callinectes sapidus) and design of a behavioral test for migrating larval decapods. One area originally proposed, the impact upon marine plants (Zostera), has been indefinitely postponed until the chemical definition of chlorination by-products is more complete.

Investigations carried out at Bears Bluff Field Station include:

1. Variations of "chlorine demand" in natural waters over the tidal cycle (Crane et al., manuscript in preparation). In this analysis, chlorine demand variations are clearly related to tidal stage, with a dozen parameters being tested statistically to clarify the role of principal factors and their interactions.

2. "Free Adenosine Tri-phosphate (ATP)" from the sea water system, marine ecosystem testing units, and marsh ponds have been tested (Erickson et al., in manuscript). The marine ecosystem testing units (METU) provided nonchlorinated controls and three levels of low chlorination for comparison. The ATP levels were clearly depressed according to the chlorination level, with the implication that productivity may be affected (Gentile et al., 1976).

3. Several studies have concerned the effects of chlorination upon early life history stages of fishes (Middaugh et al., 1977; Middaugh et al., in manuscript).

   In studies of the striped bass (Morone saxitilis), percent of egg hatchability, incipient LC_{50} bioassays, histopathological effects, and behavioral avoidance were used to test response to chlorination (Middaugh et al., 1977). Developing embryos were exposed

8-9 hr after fertilization until normal time of hatching to a series of low levels of continuous chlorination rates, as low as 0.01 mg/ℓ. Histological examination of surviving 30-day-old juveniles from incipient LC_{50} bioassays demonstrated gill and pseudobranch damage in individuals at 0.21 to 2.36 mg/ℓ in chlorination-induced oxidant levels.

Avoidance tests using 24-day-old juvenile striped bass exhibited experimentally reproducible responses at chlorination-induced oxidant levels between 0.29-0.32 mg/ℓ, and 0.79-0.82 mg/ℓ; however, they failed to respond to levels between 0.16-0.18 mg/ℓ.

4. The marine fish spot (*Leiostomus xanthurus*) was tested in the juvenile stages (Middaugh *et al.*, in manuscript). The spot showed a temperature-dependent avoidance to chlorination. At test temperatures of 15° and 20°C, fishes exhibited avoidance at lower chlorination levels than during tests at 10°C. Exposure of spot previously acclimated at 13°C to simultaneous stresses of chlorination and temperature increases (Δ+ 5°, 10°, 13°C) exhibited increased sensitivity at the highest temperature.

5. Factorial analyses were performed using different developmental stages of the mummichog (*Fundulus heteroclitus*) at four chlorination levels, and three temperature levels during four time intervals (Middaugh *et al.*, in manuscript). These efforts showed that 1 to 7-day-old larvae were much more sensitive than embryos. The chorionic membrane of the egg of this species seems to afford protection to the embryonic stages.

6. The impacts of low-level chlorination (<1.0 ppm) upon the condition of the oyster (*Crassostrea virginica*) are currently being tested. Survival, feeding response, fecal pellet production, tissue respiration, reproductive success, and larval response are being investigated. It is interesting to note that larval oysters settle only in non-chlorination controls in METU tests (Scott and Middaugh, in manuscript; Scott and Laurence, in manuscript).

7. Larval organisms entrained in the sea water system are settled in 38 ℓ METUs and are being systematically sampled (Davis *et al.*, 1977). Three levels of continual chlorination are tested (nominal levels of 0.50, 0.25, and 0.125 ppm). These microcosms are maintained outdoors and exposed to natural conditions of lighting and precipitation. Distinct effects have been seen in algal populations, sediment characteristics, and among selected invertebrates (e.g., oysters and barnacles) to chlorination levels.

COORDINATION OF MARINE CHLORINATION RESEARCH RESULTS

It is obvious from the broad spread of the aspects of chlorination in marine waters that the needs of EPA and other decision makers cannot be met solely by a modest research program and a collection of well-designed scientific projects. The key is comparison of results and communication among a wide variety of researchers. We have been fortunate that two symposia are timed to accommodate the rapid progress in the marine section of chlorination research. The first symposium, Environmental Impact of Water Chlorination held in 1975 has been published (R. Jolley, ed., 1976), and the second symposium is scheduled for early November 1977. These symposia will serve to bring the total chlorination research and application literature up to date. The forthcoming symposium serves as a milestone for publication of the scientific outcomes of each of the EPA/OEMI extramural grants and Gulf Breeze Environmental Research Laboratory/Bears Bluff Field Station in-house projects as well. The EPA/OEMI workshop held at Chesapeake Biological Laboratory provided an intermediate step to maintain communication among the "marine" section of chlorination research. It is clear that research activities at this point have proceeded from the initial emphasis upon toxicological effects of sea water chlorination per se to the definition of the roles and effects of various by-products resulting from chlorination practices.

Other oxidizing agents have been proposed as replacements for chlorination, including bromochloride and ozone. However, in marine waters, we now know that release of bromine is the key feature, and it is clear bromine is released by both of these (alternative) chemical oxidizing agents. Therefore, chlorination practices must be carefully examined on the basis of real need.

Practices in England are distinctly different than in the United States (Coughlin and Whitehouse, 1977). Adjustment of chlorination to achieve avoidance by fouling organisms would drastically reduce current chlorination levels in specific cases. In certain tropical areas, chlorination has been dropped altogether, since in these situations the thermal increase alone prevents biological fouling. The role of dechlorination must be carefully examined as an option, where antifouling is demonstrated to be uncontrollable by other means. Elimination of power plant chlorination may be achieved by simply requiring the need for chlorination to be clearly demonstrated as the only option available.

Chlorination for disinfection of effluents from municipal treatment plants entering marine environments seems to be a need based on myth or fear of pathogens, since it is principally in the continental United States that this destructive practice is maintained. Furthermore, it is far from certain that chlorination, in fact, controls viral pathogens nearly as well as the indicator bacterium *E. coli* used to assess municipal effluents. For thousands of years, man in various places and among many cultures has realized that fresh water occasionally transmits disease by direct contact (other than drinking), but rarely are marine waters implicated in pathogenic transmission. The idea that we must disinfect well-processed municipal effluents entering marine waters is basically absurd. Standards must address the problem rather than continue to perpetuate a convenient myth.

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DISCUSSION

From the floor: I have to take exception to that statement you just made. Dr. Cavelli has been conducting an epidemiological study of bathers at Rockaway and Coney Island. He has been correlating the incidence of gastrointestinal upset with coliform levels and other indicators in the swimming water.

From the floor: Would funding of research to derive engineering approaches to dechlorination be a wiser policy for the EPA than an ecotex program?

Dr. Davis: Originally, we were studying this purely from the point of view of the oxidizing effect on the organism. I think, at this point, that we have demonstrated that this issue is secondary to assessing the pathways of the induced halogenated by-products. I am not sure that dechlorination is the only alternative.

Dr. Carpenter: There was some preliminary work at the University of Miami showing that ferrates are effective biocides and that the end product is just ferric iron, a relatively innocuous material to return to the environment.

Dr. Davis: I think the problem with the pathogens and incidence of disease in recreational waters is that you are trying to correlate it with coliform. Until we correlate it with the actual pathogen, which is the basis of the problem, we are always going to be whistling in the dark.

Dr. Baumgartner: I think that dechlorination will probably work for some power plants. For municipal sewage discharges, it depends on how well the sewage is treated, how the dechlorination step is done, and how soon it is done. If chlorinated organics are formed, some of the conventional dechlorination steps are just going to remove the free chlorine.

Dr. Davis: You are just going to reduce free chlorine and not affect the halogen.

Dr. Baumgartner: If it is a poorly treated sewage, the chlorinated organics, in the long run, may be a much greater problem than the free chlorine residuals in the marine environment.

Dr. Hergott: Dechlorination in power plants is not easy, because some kind of contact time is required. At the power plants in California, there is approximately 1/2 min of flow time from the condensors to the outflow, which is not long enough.

Dr. Davis: At least, you achieve a lot of mixing during that time. Are the rates for reaction of dechlorination available?

Mr. Hergott: It depends on the form of the residual.

Dr. Davis: It does require reexamining each situation and redesign. On the other hand, we are using a design basically set up by the municipal treatment supply industries, which predicate everything on a chlorine residual. There is no real attempt on their part to find out the necessary level of chlorination; or what they are preventing; and at what time of year; and how much is needed to achieve it.

Dr. Baumgartner: Maybe there is enough evidence to say that power plants should not use chlorine or should use it in such a way that the residual is 0.001.

Dr. Riley: Are you running any studies when you chlorinate at higher temperatures? For example, the cooling tower temperatures?

Dr. Davis: We are not trying to assimilate cooling tower processes. The other part of your question is answered by the fact that, in the outdoor exposure, we are monitoring the seasonal temperature and the temperature of the sea water continually. In the laboratory, where we are studying exposures of the individual species, we are working at higher temperatures.

Dr. Riley: What are your future plans for looking at some of these chlorinated organic compounds with regard to toxicity to marine organisms?

Dr. Davis: There seems to be a distinct uptake by fishes, at least, of bromoform. We rather expected bromoform to be volatile and disappear like your light oil fraction. We can readily identify the other compounds.

From the floor: I have one question. Have you looked at any pelagic fish eggs, relative to the benthic ones?

Dr. Davis: To compare them? No. If you are thinking about higher sensitivity of a pelagic fish egg, I would agree.

From the floor: We found that there is a difference, for example, between the menidia and spot, with regard to count. One will affect the development, and the other one will not.

Dr. Davis: In this case, the menidia is probably resistant. EPA's alternative prime fish species lays its eggs intertidally.

From the floor: Have you heard anything about this chlorine flux monitor that may be produced?

Dr. Davis: In essence, I do not think it is any different than an aperometic titrator.

Dr. Carpenter: The flux monitor has the same basic chemistry as the titrator, only the time of reaction is probably 10 or 15 sec. between the point of injection of the potassium iodide and the electrodes. However, you are just not going to get quantitative results. You will severely underestimate the actual residual oxide with the flux monitor.

Dr. Riley: What is the GC mass spectroscopy technique that has been used to identify these chlorinated organics?

Dr. Carpenter: Basically, it is the Hewlitt-Packard system.

Dr. Anderson: You mentioned something about the eggs being perhaps the most resistant or most sensitive.

Dr. Davis: More resistant than newly hatched. We are examining the stage life history against different temperatures and different doses. The particular case with coreanic membrane just indicates relative sensitivity at life history stage.

Dr. Anderson: That is certainly consistent with the hydrocarbon findings. However, we found that it was not necessarily the coreanic membrane, because I had one person who actually dissected the membrane off of a number of embryos and the tolerance was the same.

Dr. Davis: What was the organism?

Dr. Anderson: It was a fungilus, either a heteroquitous or a grandis.

Dr. Davis: We are trying this with a self-fertilizing hermaphrodite, to see if we can get successful embryological development without the coreanic, and compare that. I am interested that somebody has, at least, done it.

Dr. Carpenter: I just wanted to make one comment concerning new regulations. If regulations are arbitrarily restrictive, you are driving people to some other technology, probably mechanical, à la Amortat, which could increase the copper emission rate. So, we need to examine whether we would rather have copper or chlorine before deciding what should be done. I think it is a little bit early to determine the magnitude of chlorine environmental impact.

POWER PLANT COOLING WATER CHLORINATION IN NORTHERN CALIFORNIA

S. Hergott, D. Jenkins, and J.F. Thomas*

Power plants require large volumes of cooling water to carry away waste heat. If fouling is not controlled, bacterial and algal slimes attach themselves to the walls of the piping and decrease the heat transfer across the condensers. The most economic method currently in use to control fouling is the addition of chlorine to the cooling water.

Chlorine presents problems when its toxic effects carry over into the receiving waters. Brungs (1973) conducted a comprehensive review of the literature dealing with toxicity of both continuous and intermittent chlorine treatment to fresh water fish. Based on these studies, he recommended intermittent chlorine limits in the discharge of power plants that vary from 0.2 mg/ℓ to 0.04 mg/ℓ for up to 2 hr per day, depending on the degree of protection desired. Basch and Truchan (1976) studied the effect of intermittent chlorination on brown trout (*Salmo trutta*) at five Michigan power plants. They found the 48 hr total residual chlorine intermittent concentrations lethal to 50% (ILC-50) of the brown trout to range from 0.14 to 0.17 mg/ℓ and 0.18 to 0.19 mg/ℓ for fish exposed to two and four 30 min chlorination cycles, respectively. Mattice and Zittel (1976) conducted a comprehensive review of the literature dealing with the toxicity of chlorine to both fresh water and marine organisms. Based on this research, they determined both acute and chronic toxicity thresholds and concluded that marine organisms appear more susceptible to acute doses of chlorine, whereas fresh water organisms appear more susceptible to chronic concentrations.

The increasing awareness of the toxicity of chlorine residuals has prompted regulatory control of chlorine discharges in power plant cooling waters. The Environmental Protection Agency (EPA) has established free chlorine discharge guidelines of 0.5 mg/ℓ maximum and 0.2 mg/ℓ average concentrations for up to 2 hours per day from any one unit (1974). The California Central Coast Regional Water Quality Control

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Board has set daily maximum limits of 0.02 mg Cl_2/ℓ undissociated free available chlorine and 0.1 mg Cl_2/ℓ total available chlorine in power plant discharges (1976). The San Francisco Bay Regional Water Quality Control Board has set even stricter limitations of an instantaneous maximum chlorine residual of 0.0 mg/ℓ (1976).

This study was conducted to determine the nature, levels, and persistence of chlorinated compounds in the discharges of five power plants in northern California. In-plant studies were conducted to understand the demands for chlorine in the cooling water system and to determine the levels reaching the receiving waters. Decay studies conducted at the outfalls, together with measurements of oxidant residuals in the receiving water, provide a base for estimating the persistence and zone of influence of the chlorinated compounds.

MATERIAL AND METHODS

We describe below the power plants and their location, its chlorination practices, field study procedures, and analytical methods and equipment.

Power Plant Location and Description

Exhibit 1 is a map showing the location of the five power plants studied. Water quality varied from fresh at Contra Costa, to marine at Moss Landing.

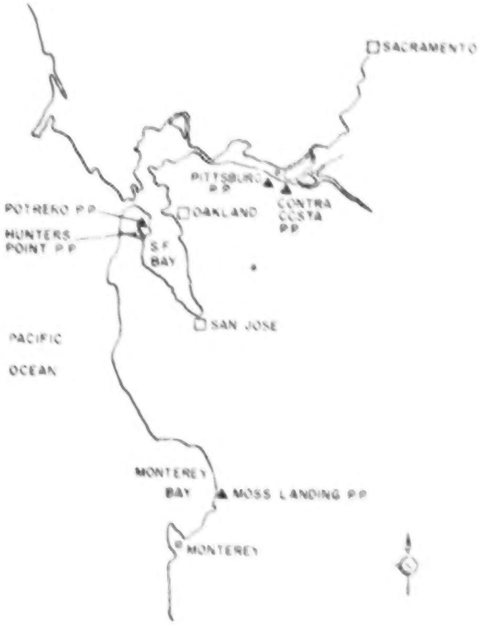


EXHIBIT 1: POWER PLANT LOCATIONS

A summary of the operating data for each plant is presented in Exhibit 2. All the plants have the capability of burning either fuel oil or natural gas. The plants rarely operated at maximum capacity. Usually, the newer, more efficient units in any plant operated continuously, and the older units were used during peak demand periods and during periodic shutdowns of the other units.

EXHIBIT 2: POWER PLANT OPERATING DATA

Power Plant Plant	Gross Generating Capacity (MW)	No. of Units	Source of Intake Water	Cooling Water Flow Rate*	
				(m^3/sec)	(gpm)
Contra Costa	1300	7	San Joaquin River	43.2	684 000
Pittsburg	2060	7†	Suisun Bay	45.4	720 000
Hunters Point	400	3	San Francisco Bay	18.2	288 000
Potrero	330	3	San Francisco Bay	16.5	262 000
Moss Landing	2110	7	Monterey Bay	63.1	1 000 000

* All units in operation.

† Unit 7 cooled by a spray canal.

Plan diagrams of the once-through cooling water systems at each of the power plants are presented in Exhibits 3 to 7. Cooling water is diverted from its source and pumped through the cooling system by circulating water pumps located at the intake structures. The cooling water first passes through bar racks, which prevent large objects from entering the system. Smaller objects are removed by traveling screens, which rotate periodically and are cleaned with high pressure water.

Heat exchange between cooling water and superheated steam occurs in the condensers, which consist of bundles of 22.2 mm (7/8 in.) or 25.4 mm (1 in.) diameter aluminum-brass or copper-nickel alloy tubes. There are generally two condensers per unit. The heated flows of cooling water combine immediately after the condensers and are discharged to the receiving water at the outfall structure.

In Exhibits 3 to 7, cooling water sampling locations are indicated for each power plant. Cooling water could generally be sampled at the condenser inlets (COND-IN), condenser outlets (COND-OUT), and at outfall manholes (OUT). Convenient sample taps had been set up at the COND-IN, because this was where oxidant residuals were routinely measured by plant operating personnel. Although taps generally existed or were readily installed, sampling at the COND-OUT was more difficult, because samples had to be pumped out against a vacuum of about 25.4 cm (10 in.)

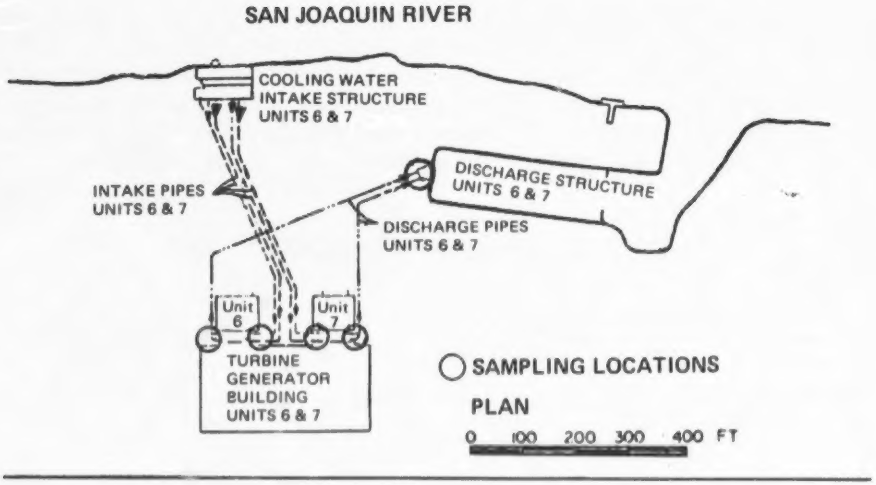

SAN JOAQUIN RIVER
COOLING WATER INTAKE STRUCTURE UNITS 6 & 7
INTAKE PIPES UNITS 6 & 7
DISCHARGE STRUCTURE UNITS 6 & 7
DISCHARGE PIPES UNITS 6 & 7
Unit 6
Unit 7
TURBINE GENERATOR BUILDING UNITS 6 & 7
SAMPLING LOCATIONS
PLAN
0 100 200 300 400 FT

EXHIBIT 3: CONTRA COSTA POWER PLANT

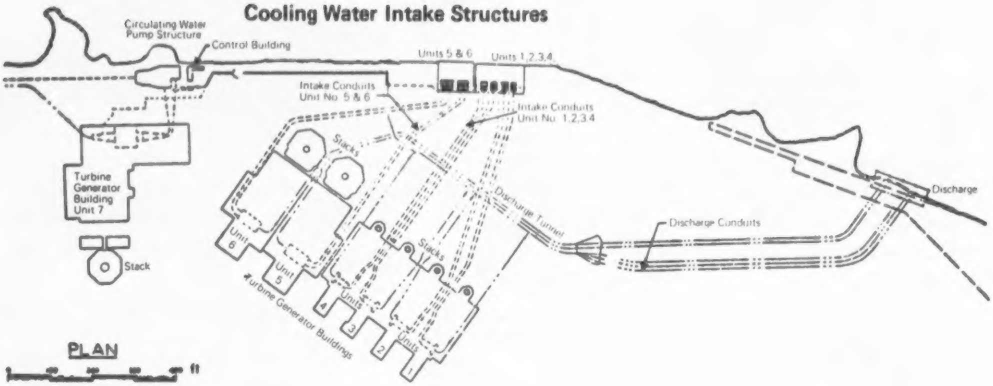

Cooling Water Intake Structures
Circulating Water Pump Structure
Control Building
Units 5 & 6
Units 1,2,3,4
Intake Conduits Unit No. 5 & 6
Intake Conduits Unit No. 1,2,3,4
Stacks
Turbine Generator Building Unit 7
Stack
Discharge Tunnel
Discharge Conduits
Discharge
Unit 6
Unit 5
Units 4 3
Units 2 1
Turbine Generator Buildings
PLAN
ft

EXHIBIT 4: PITTSBURG POWER PLANT

EXHIBIT 5: HUNTERS POINT POWER PLANT

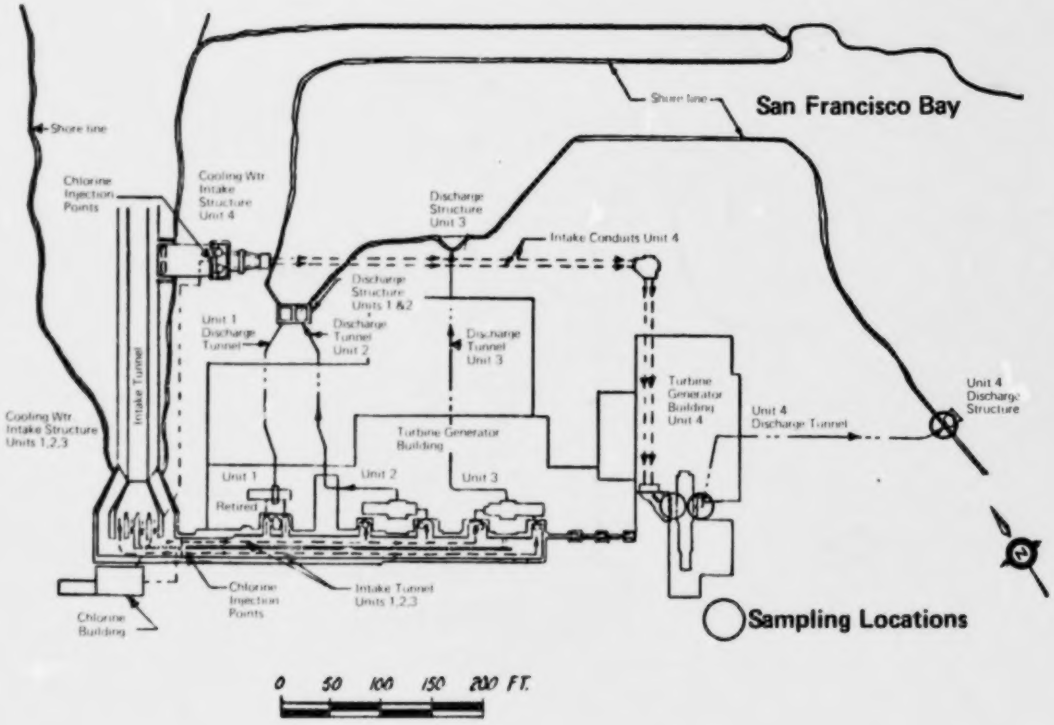
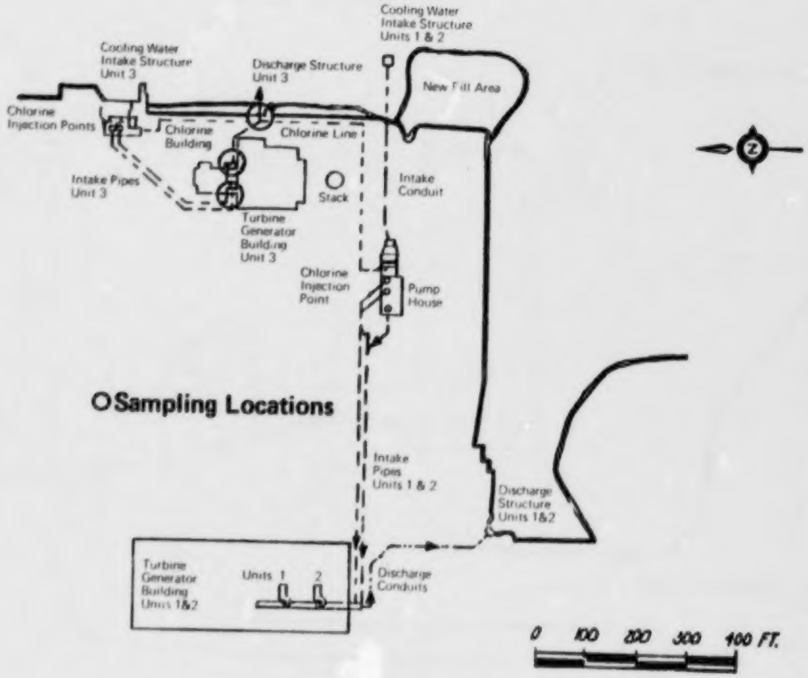

San Francisco Bay
Shore line
Shore line
Chlorine Injection Points
Cooling Wtr Intake Structure Unit 4
Discharge Structure Unit 3
Intake Conduits Unit 4
Discharge Structure Units 1 &2
Unit 1 Discharge Tunnel
Discharge Tunnel Unit 2
Discharge Tunnel Unit 3
Intake Tunnel
Cooling Wtr Intake Structure Units 1,2,3
Turbine Generator Building
Turbine Generator Building Unit 4
Unit 4 Discharge Tunnel
Unit 4 Discharge Structure
Unit 1
Retired
Unit 2
Unit 3
Chlorine Building
Chlorine Injection Points
Intake Tunnel Units 1,2,3
Sampling Locations
N
0 50 100 150 200 FT.

EXHIBIT 6: POTRERO POWER PLANT


Cooling Water Intake Structure Units 1 & 2
Cooling Water Intake Structure Unit 3
Discharge Structure Unit 3
New Fill Area
Chlorine Injection Points
Chlorine Building
Chlorine Line
Intake Pipes Unit 3
Stack
Intake Conduit
Turbine Generator Building Unit 3
Chlorine Injection Point
Pump House
Sampling Locations
Intake Pipes Units 1 & 2
Discharge Structure Units 1&2
Turbine Generator Building Units 1&2
Units 1 2
Discharge Conduits
0 100 200 300 400 FT.

of mercury. Outfall manholes generally existed as part of a discharge structure so that samples taken from these manholes were representative of the water being discharged to the receiving water. At Moss Landing, Units 6 and 7 discharged into Monterey Bay, 242 m (800 ft) offshore, at a depth of 6.1 m (20 ft). A sampling point approximately midway between the condensers and the outfall was furnished by International Shellfish Enterprises, Inc.

Chlorination Practices

Chlorine is injected intermittently into the cooling water flow to control slime-causing organisms. In Pacific Gas and Electric Company (PG&E) plants, liquid chlorine is withdrawn from 1-ton cylinders, evaporated, and injected into a water line to produce a concentrated chlorine solution. This solution flows to the point of injection in a well behind the traveling screens and in front of the circulating water pumps. Oxidant residuals are routinely measured at the COND-IN, and the chlorine injection rate is adjusted to give the desired residual. These adjustments are rarely needed on a daily basis; however, the residual monitoring is important to ensure proper operation of the chlorinators. Chlorinators are somewhat unreliable when subjected to the frequent startups and shutdowns encountered in intermittent chlorination.

A summary of chlorination practices for the newest units at each of the five PG&E power plants is presented in Exhibit 8. The newest units were chosen for study because they were the largest and most frequently operated. A great deal of variation in frequency, duration, and levels of chlorination existed. Frequency varied from 1/week to 4/day and was changed during the year to meet changing demands. Duration of chlorination ranged from 15 to 40 min. All the plants seek to maintain a 0.5 mg/ℓ residual at the COND-IN except for Moss Landing, where a 1.0 mg/ℓ residual for a shorter duration was used. Hunters Point was the only plant to measure a free residual and use it as their criterion for dosing. Until recently, the orthotolidine method was used at all the plants, because it was the most portable and the easiest for the operators to learn and use. All the plants are currently using or are in the process of changing over to the amperometric titration method.

Field Study Procedures

Field studies were conducted at four of the power plants. Since no convenient sampling point existed near the outfall, no studies were conducted at the Pittsburg plant. Studies were conducted during normal chlorination cycles and were designed to monitor existing chlorination practices; therefore, no changes were made in chlorine injection rate, duration, or frequency of chlorination cycles to accommodate this work.

At the beginning of the study, oxidant residuals were determined solely by the amperometric method. During the course of the investigation, the DPD-FAS titrimetric method was added in an attempt to distinguish between bromine and chlorine residuals.

EXHIBIT 7: MOSS LANDING POWER PLANT

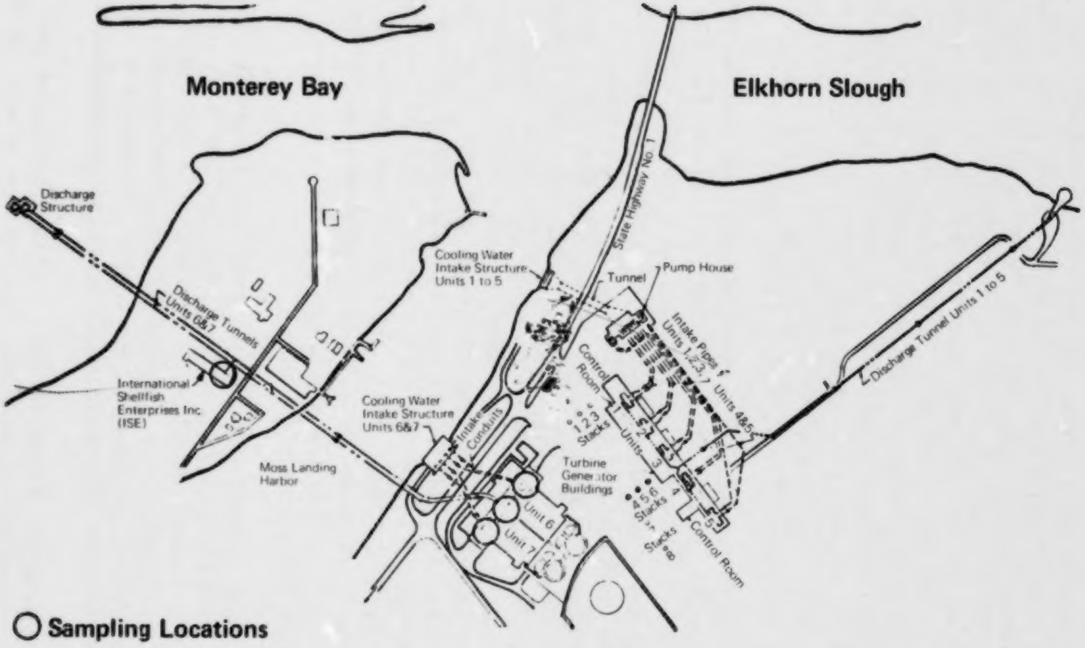

Monterey Bay
Elkhorn Slough
Discharge Structure
Discharge Tunnels Units 6&7
International Shellfish Enterprises Inc (ISE)
Moss Landing Harbor
Cooling Water Intake Structure Units 1 to 5
Cooling Water Intake Structure Units 6&7
Intake Conduits
State Highway No 1
Tunnel
Pump House
Intake Pipes Units 1,2,3, 7 Units 4&5
Control Room
1 2 3 Stacks
Units 1 2 3 4 5
Turbine Generator Buildings
4 5 6 Stacks
Stacks
Control Room
Unit 6
Unit 7
Discharge Tunnel Units 1 to 5
○ Sampling Locations

EXHIBIT 8: CHLORINATION PRACTICES AT PACIFIC GAS AND ELECTRIC PLANTS

Power Plant	Unit(s)	Chlorination Schedule		Criteria at COND-IN (mg Cl_2/ℓ)	Analytical Method	
		Frequency	Duration (min)		Nov. 1975	Current/Future
Contra Costa	6,7	1/week	40	TOR=0.5	Amp. Tit.	Amp. Tit.
Pittsburg	5,6	Varies with season		TOR=0.5	OT*	Amp. Tit.
		Min-1/week Max-1/day	30 30			
Hunters Point	4	4/day	30	TOR=0.5†	Amp. Tit.	Amp. Tit.
Potrero	3	2/day	30	TOR=0.5†	OT*	Amp. Tit.
Moss Landing	6,7	1/day	15	TOR=1.0	OT*	OT/Amp. Tit.

* OT = Orthotolidine Method.

† Lowered to 0.5 mg/ℓ in January 1977.

Cooling water samples were collected for the determinations of the water quality parameters listed in Exhibits 9-11. At the COND-IN, triplicate 1ℓ grab samples were taken before, during, and after chlorination, both from a condenser being chlorinated and from one that was not. Samples were taken at the outfalls during chlorination. To destroy oxidant residuals, a small excess of sodium arsenite was added to all samples taken during chlorination. No interference from this procedure was found in any of the analytical methods, but there was some evidence that the sodium arsenite did not always react instantaneously with the oxidant residual. This would probably result in lower concentrations of NH_3-N and organic nitrogen in those samples taken during chlorination.

Decay studies were conducted on chlorinated cooling water collected at the outfalls. Two 8ℓ (2.1 gal) containers lined with polyethylene bags were filled with cooling water. One container was covered to exclude sunlight. No effort was made to keep temperatures constant. Oxidant residuals were determined by the amperometric titration method in both containers over a period of approximately 60 min.

Total oxidant residuals were determined in receiving waters by forward amperometric titrations at all plants except Moss Landing. In this plant, samples were taken for later titration, using the back-titration method for free oxidant residual. This method was necessary because the turbulence and wave action at the outfall caused the microammeter needle of the titrator to vibrate and made the end-point difficult to read. Receiving water samples were generally taken at the surface, although some samples were taken at depths of 1, 2, and 3m (3.3, 6.6, and 9.9 ft). Sampling point locations were determined by reference to onshore features and in some cases by a rope attached to the outfall structure.

Analytical Methods and Equipment

To measure oxidant residuals, two methods are used, amperometric titration and DPD-FAS titrimetric method.

1. Amperometric titration. Oxidant residuals were determined amperometrically using Fischer and Porter (Model 17T1010) portable titrators. Total oxidant residuals (TOR) were determined by a direct titration according to standard methods (1974). TOR could be determined in approximately 1.5 min by applying some suggestions by Manabe (1974). These included the use of a graduated cylinder modified to measure a 200 mℓ sample quickly and the addition of up to 90% of the titrant before turning on the stirrer. The back-titration procedure described by White (1972) for the determination of free oxidant residual (FOR) was adopted because of an unstable end-point experienced during a direct titration. The term "oxidant residual" has been used rather than "chlorine residual" because the amperometric method measures bromine and iodine residuals in addition to chlorine residuals.

EXHIBIT 9: WATER QUALITY AND TIDAL DATA AT THE CONTRA COSTA POWER PLANT

Date/Sample	NH_3-N mg/ℓ	Org-N mg/ℓ	Cl^- mg/ℓ	Br^- mg/ℓ	S.S. mg/ℓ	pH	Time of Study	Tidal Data Time	Tidal Data Ht/ft
20 Jan 1976									
#11 COND-IN DUR	0.04*	0.13	50		22		0930-1200	Hi 0602	3.9
#14 COND-IN DUR			50		19			Lo 1213	1.0
10 Feb 1976									
#13 COND-IN BEF	0.11*	0.29	563	1.2	42		0930-1120	Hi 1039	4.0
#13 COND-IN DUR	0.09*	0.21	648	1.6	30			Lo 1841	0.2
#13 COND-IN AFT	0.10*	0.20	901	1.9	35				
16 Jun 1976									
#13 COND-IN BEF	0.091	0.42	1130	3.9	111	7.5-	1010-1100	Hi 0646	3.8
#13 COND-IN DUR(+)[†]	0.066	0.40	1019	3.6	118	7.7		Lo 1415	0.2
#13 COND-IN DUR(-)**	0.078	0.43		3.5	128				
7 OUT DUR	0.082	0.41		3.4	114				
#13 COND-IN AFT	0.078	0.36	746	2.8	88				
14 Sept 1976									
#11 COND-IN BEF		0.33	1032	3.9	34	7.7-	1030-1250	Hi 0835	3.0
#12 COND-IN DUR(+)[†]	0.019	0.31			37	8.0		Lo 1403	1.4
#11 COND-IN DUR(-)**	0.012	0.33	913	3.5	34				
6 OUT DUR	0.009	0.34			32				
#13 COND-IN AFT	0.017	0.32	761	3.4	41				

* Determined by nesslerization.

[†] Samples taken during chlorination cycle from the condenser being chlorinated.

** Samples taken during chlorination cycle from a condenser not being chlorinated.

EXHIBIT 10: WATER QUALITY AND TIDAL DATA AT THE HUNTERS POINT AND POTRERO POWER PLANTS

Date/Sample	NH_3-N mg/ℓ	Org-N mg/ℓ	Cl^- g/ℓ	Br^- mg/ℓ	S.S. mg/ℓ	pH	Time of Study	Tidal Data	
								Time	Ht/ft
Hunters Point									
16 Dec 75									
COND-IN DUR	0.06*	0.33	15.4		14		1100-1145		
17 May 76									
COND-IN BEF	0.134	0.18	18.2		27	7.0	1040-1120	Hi 1655	5.8
COND-IN DUR	0.047	0.08	17.7		19			Lo 0959	-1.1
OUT DUR	0.036	0.03			19				
COND-IN AFT	0.119	0.14	17.9		21				
26 Aug 76									
COND-IN BEF	0.128	0.34	18.7	72	31	7.5	1040-1120	Hi 1422	7.1
COND-IN DUR	0.037	0.28			28			Lo 0813	1.6
OUT DUR	0.024	0.28			28				
COND-IN AFT	0.140	0.36	18.4	73	27				
Potrero									
23 Feb 76									
N. COND-IN BEF	0.055	0.27	16.8	67	13		2100-2215	Hi 2026	4.8
N. COND-IN DUR	0.090	0.20	16.5	71	11			Lo 2509	2.7
OUT DUR	0.032	0.21	16.5	70	12				
N. COND-IN AFT	0.075	0.26	16.4	71	10				
4 Mar 76									
N COND-IN BEF	0.118	0.20	16.7	63			2100-2215	Hi 2625	5.7
N COND-IN DUR	0.059	0.16	16.8	64				Lo 2000	1.6
OUT DUR	0.077	0.21							
S COND-IN AFT	0.104	0.17	16.8	66					

* Determined by nesslerization.

EXHIBIT 11: WATER QUALITY AND TIDAL DATA AT THE MOSS LANDING POWER PLANT

	NH_3-N	Org-N	Cl^-	Br^-	S.S.	pH	Time of Study	Tidal Data	
Date/Sample	mg/ℓ	mg/ℓ	g/ℓ	mg/ℓ	mg/ℓ			Time	Ht/ft
12 Apr 76									
7-1 COND-IN BEF	0.096	0.20	19.0	78	19	7.9-8.0	1240-1330	Hi 0838	4.7
7-2 COND-IN DUR(+)*	0.021	0.09	18.7	82	22			Lo 1438	0.5
7-1 COND-IN DUR(-)†	0.114	0.19	18.8	81	21				
7-2 COND-IN AFT	0.137	0.25	18.5	82	21				
19 Apr 76									
7-1 COND-IN BEF	0.058	0.14	18.9	68	31	7.8	1300-1350	Hi 1533	4.1
7-2 COND-IN DUR(+)*	0.005	0.08	19.0	70	14			Lo 0840	-0.6
7-1 COND-IN DUR(-)†	0.033	0.12	18.8	67	15				
7 ISE DUR	0.016	0.10			19				
7-2 COND-IN AFT	0.037	0.04	19.1	74	20				
14 Jul 76									
6-1 COND-IN BEF	0.046	0.19	18.6	82	12	8.1-8.2	1300-1400	Hi 1403	4.8
6-2 COND-IN DUR(+)*	0.000	0.19			14			Lo 0729	-0.4
6-1 COND-IN DUR(-)†	0.038	0.19			8				
6 ISE DUR	0.010	0.19			12				
6-1 COND-IN AFT	0.047	0.19	19.1	80	8				
22 Jul 76									
6-1 COND-IN BEF	0.072	0.30	18.8	78	15	8.0-8.1	1300-1350	Hi 0908	3.5
6-2 COND-IN DUR(+)*	0.010	0.19			9			Lo 1327	2.8
6-1 COND-IN DUR(-)†	0.120	0.30			11				
6-1 COND-IN AFT	0.093	0.26	18.9	74	10				
5 Aug 76									
6-1 COND-IN BEF	0.074	0.22	18.8	73	23	8.2-8.3	1300-1400	Hi 0826	3.8
6-1 COND-IN DUR(+)*	0.016	0.17			18			Lo 1307	2.7
6-2 COND-IN DUR(-)†	0.072	0.21			18				
6 ISE DUR	0.045	0.17			16				
6-1 COND-IN AFT	0.074	0.23	19.1	70	18				
23 Sept 76									
6-1 COND-IN BEF	0.052	0.19	18.7	70	3	8.9	1300-1350	Hi 1044	5.4
6-1 COND-IN DUR(+)*	0.045	0.25			5			Lo 1656	0.3
6-2 COND-IN DUR(-)†	0.071	0.22			4				
6 ISE DUR	0.017	0.16			2				
6-1 COND-IN AFT	0.103	0.30	18.6	67	4				

* Samples taken during chlorination cycle from the condenser being chlorinated.

† Samples taken during chlorination cycle but from a condenser not being chlorinated.

2. DPD-FAS titrimetric method. The DPD-FAS titrimetric method of Palin (1957, 1961, 1975) was used to distinguish chlorine and bromine residuals. Three separate titrations give: free chlorine plus free and combined bromine; combined chlorine; and free and combined bromine. The separate determination of free and combined bromine is not possible.

Organic nitrogen, chloride, bromide, and suspended solids were all determined according to standard methods (1976). Ammonia-nitrogen was determined by the phenolhypochlorite method of Solorzano (1969). This method was found to be well suited for fresh, marine, and estuarine waters by Zadorojny et al. (1973).

RESULTS

In this section, we describe the results of our in-plant analyses and decay and recovery water analyses.

In-Plant Studies

Persistence and decay of oxidant residuals at the outfalls and in the receiving waters can best be explained after the nature and disappearance of the residual inside the plants are understood. The in-plant studies were conducted to establish the nature of the oxidant residual and to gain an understanding of the demands in the cooling water systems at each of the power plants. Each plant was studied separately because of variations in water quality, chlorine dosage, frequency and duration of chlorination, and flow time through the system.

In-plant studies were conducted over a period of 1 year to be inclusive of varying water quality conditions. Typical results at each of the four plants studied are presented in Exhibits 12 to 15. The graphs show the temporal variation of oxidant residual during a chlorination cycle. Also shown is the chlorine dose calculated on the basis of the chlorine injection rate determined by power plant personnel and the cooling water flow rate. The calculated dose may not be precise, because the chlorine injection rate was difficult to read accurately. In addition, the only value usually known for the cooling water flow rate was the original design value.

Contra Costa Power Plant The results of the in-plant field studies conducted at the Contra Costa power plant are summarized in Exhibit 16. Water quality and tidal data corresponding to most of these studies are presented in Exhibit 9.

A combined residual of from 0.10 to 0.40 mg Cl_2/ℓ formed by the time the cooling water reached the COND-IN (approximately 1.2 min of flow time) and generally remained unchanged at the outfall when the dilution effect was taken into account. It appeared that the FOR which

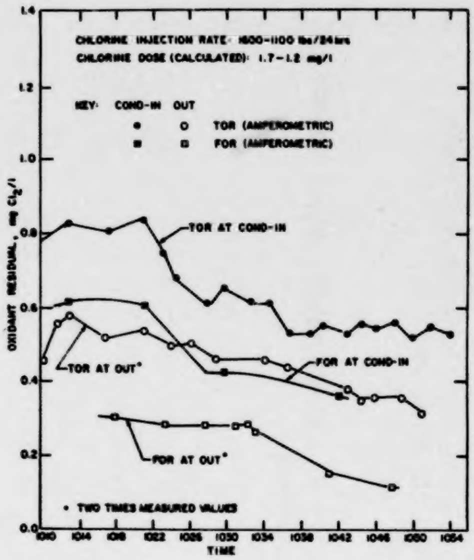


EXHIBIT 12: IN-PLANT STUDY AT THE CONTRA COSTA POWER PLANT, 6 JUNE 1976, UNIT 7, CONDENSER NO. 13

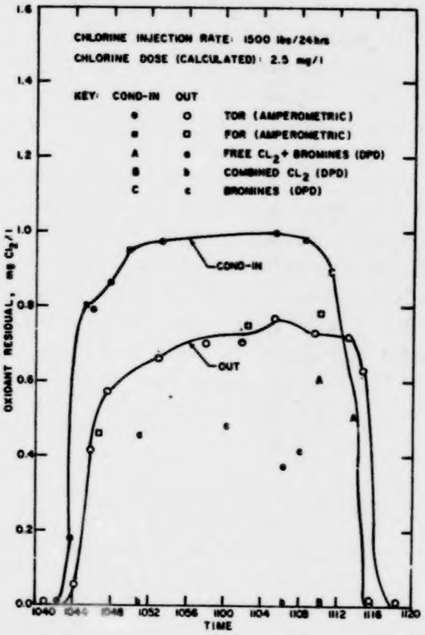


EXHIBIT 13: IN-PLANT STUDY AT THE HUNTERS POINT POWER PLANT, 26 AUGUST 1976, UNIT 4

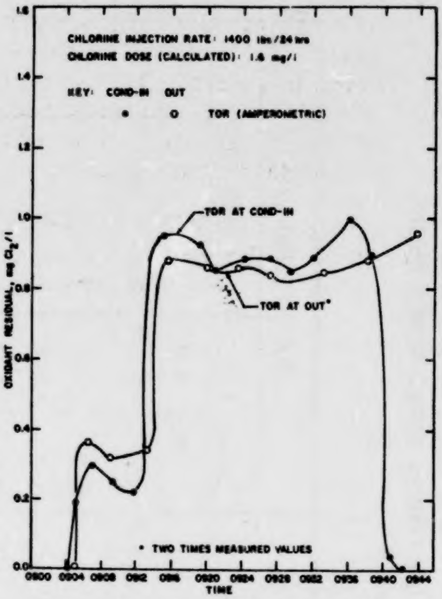


EXHIBIT 14: IN-PLANT STUDY AT THE POTRERO POWER PLANT, 4 MARCH 1976, UNIT 3, N. CONDENSER

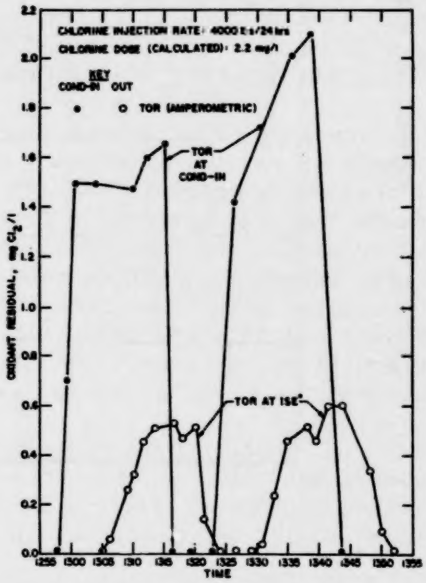


EXHIBIT 15: IN-PLANT STUDY AT THE MOSS LANDING POWER PLANT, 19 APRIL 1976, UNIT 7

EXHIBIT 16: SUMMARY OF FIELD RESULTS AT CONTRA COSTA POWER PLANT
UNITS 6 and 7

Date	Cl_2 Dose Calculated	Condenser-Inlet					Outfall Manhole†				
		Amp. Tit.		DPD-FAS*			Amp. Tit.		DPD-FAS*		
		TOR	FOR	A	B	C	TOR	FOR	A	B	C
	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$
20 Jan 76	1.6	0.75	0.45				0.18	0.03			
10 Feb 76	1.6	0.50	0.10				0.23	0.00			
16 Jun 76	1.2	0.55	0.36				0.18	0.08			
14 Sept 76	1.3	0.30	0.20	0.20	0.10	0.20	0.04	0.00	0.00	0.03	0.00

* Results of three titrations are:
A = Free chlorine plus bromine residuals
B = Combined chlorine residual
C = Bromine residuals.

† Chlorinated cooling water flow was diluted with an approximately equal volume of unchlorinated water immediately after the condenser.

disappeared accounted for most of the loss of residual between the COND-IN and the outfall.

The DPD-FAS results for the study of 14 September 1975 indicated a combined chlorine residual of 0.10 mg/ℓ at the COND-IN and 0.03 mg/ℓ at the outfall, both of which compare favorable with the amperometric titration results. The FOR of 0.20 mg Cl_2/ℓ determined amperometrically titrates as bromine residual by the DPD-FAS method since titration "A" equals titration "C."

Cooling water at Contra Costa varied from 0.3% to 6.1% sea water during the dry summer months. Most of the water quality data show the effects of tidal changes. The addition of chlorine generally caused a decrease in ammonia-nitrogen concentration, but this was more evident at the other power plants. Compared with earlier studies, the much lower combined residual detected on 14 September 1976 is explained by the very low NH_3-N concentration.

Hunters Point and Potrero Power Plants The results of the in-plant studies conducted at the Hunters Point and Potrero power plants are summarized in Exhibit 17. Water quality and tidal data corresponding to these studies are presented in Exhibit 10.

In general, FOR equaled TOR both at the COND-IN and at the outfall. An exception was the study of 17 May 1976, which detected a combined residual at the outfall of 0.13 mg Cl_2/ℓ that had formed during the approximately 0.9 min of flow time from the COND-IN.

The DPD-FAS results for the study of 26 August 1976 at Hunters Point revealed a small combined chlorine residual at the COND-IN, which the amperometric method failed to detect. The results at the COND-IN are difficult to explain since titration C should never be greater that titration A. At the outfall, a total residual of 0.45 mg Cl_2/ℓ was identified as bromine residual by the DPD-FAS method.

A TOR of 1.0 Cl_2/ℓ was desired at the COND-IN for the studies of 23 February 1976 and 4 March 1976 at the Potrero power plant. This criterion was lowered to 0.5 mg/ℓ and was the criterion during the studies on October 15 and 17. The studies of 4 March 1976 and 15 October 1976 revealed essentially no loss of oxidant residual between the COND-IN and outfall manhole, which corresponded to a flow time of approximately 0.4 min.

Cooling water at Hunters Point and Potrero varied from 79% sea water. Ammonia-nitrogen concentrations determined on samples collected at the COND-IN decreased by up to 0.091 mg/ℓ during chlorination. A decrease in organic nitrogen was also evident during chlorination.

Moss Landing Power Plant The results of the in-plant studies at the Moss Landing power plant are summarized in Exhibit 18. Water quality and tidal data are presented in Exhibit 11.

EXHIBIT 17: SUMMARY OF FIELD STUDY RESULTS AT THE HUNTERS POINT (UNIT 4) AND POTRERO POWER PLANTS (UNIT 3)

Date	Cl_2 Dose Calculated	Condenser Inlet					Outfall Manhole†				
		Amp. Tit.		DPD-FAS*			Amp. Tit.		DPD-FAS*		
		TOR	FOR	A	B	C	TOR	FOR	A	B	C
	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$	$mgCl_2/\ell$
Hunters Pt.											
16 Dec 76	1.6	0.85					0.50				
17 May 76	1.6	0.72	0.72				0.50	0.37			
26 Aug 76	2.5	1.00	1.00	0.60	0.10	0.90	0.70	0.70	0.45	0.00	0.45
19 Oct 76**	1.2	0.65	0.65				0.24	0.24			
Potrero											
23 Feb 76	2.0	1.3-1.5					0.5-0.6				
4 Mar 76	1.6	0.8-1.0					0.4-0.5				
15 Oct 76	0.9	0.73					0.35				
27 Oct 76	0.9	0.73					0.28				

* Results of three titrations are:
A = Free chlorine plus bromine residuals
B = Combined chlorine residual
C = Bromine residuals.

† Hunters Point - The cooling water flows through both condensers of Unit 4 and is chlorinated simultaneously. Potrero - Chlorinated cooling water was diluted with an approximately equal volume of unchlorinated water immediately after the condensers.

** Cooling water flows through the condensers were chlorinated separately for this study.

EXHIBIT 18: SUMMARY OF FIELD STUDY RESULTS AT THE MOSS LANDING POWER PLANT (UNITS 6 AND 7)

Date	Cl_2 Dose Calculated	Condenser Inlet					ISE Sampling Point†				
		Amp. Tit.		DPD-FAS*			Amp. Tit.		DPD-FAS*		
		TOR	FOR	A	B	C	TOR	FOR	A	B	C
	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$	$mgCl_2/ℓ$
12 Apr 76	2.2	1.35-1.65					0.15-0.18				
19 Apr 76	2.2	1.45-2.10					0.23-0.31				
14 Jul 76	2.1	1.40-1.80	1.60-1.90	1.70-2.00	0.00	1.80-2.20	0.15-0.20	0.10-0.14			
22 Jul 76	2.1	1.30-2.10	1.40-2.20	1.25-1.65	0.00	1.60-2.00					
5 Aug 76	1.3	0.80-1.20	0.90-1.35	0.50-0.80	0.05	0.70-1.20	0.05-0.15	0.05-0.10	0.08-0.11	0.05-0.10	0.03-0.05
23 Sep 76	2.1	1.00-1.70	1.00-1.80	1.10-1.70	0.00	1.70-2.20	0.24-0.40	0.30-0.40			

* Results of three titrations are:
A = Free chlorine plus bromine residual
B = Combined chlorine residual
C = Bromine residual.

† Chlorinated cooling water was diluted with an approximately equal volume of unchlorinated water immediately after the condensers.

The results generally indicate much higher oxidant residuals at the COND-IN than the 1.0 mg/ℓ desired by the plant chemist. Measurements by the amperometric and DPD-FAS methods were often on the order of two times the value determined by the plant chemist using the orthotolidine method.

At the COND-IN, FOR was generally greater than or equal to TOR. Since FOR can never be greater than TOR, an error (probably in the TOR measurement) was indicated. Approximately 1 min was required to measure out a 200 mℓ sample, transfer it to the titration jar, add the KI and pH 4 buffer, and perform the TOR titration. During this time, the oxidant residual was reacting with species in the water and decaying. On the other hand, the back-titration method for FOR fixes the concentration as soon as the sample is mixed with excess phenylarsine oxide and pH 7 buffer. Another contributing factor to this result was the flashing off of iodine during stirring in the forward titration for TOR.

The DPD-FAS results indicated little or no combined chlorine residual at the COND-IN. Some combined chlorine was indicated at the sampling point at International Shellfish Enterprises, Inc., both by the amperometric and DPD-FAS methods. However, most of the DPD-FAS results must be regarded with suspicion because of the discrepancy between titrations A and C.

Cooling water at Moss Landing varied from 95% to 99% sea water. Ammonia-nitrogen concentrations determined on samples collected at the COND-IN decreased by up to 0.075 mg/ℓ because of chlorination. A decrease of organic nitrogen during chlorination was observed in four of the six studies.

Summary The DPD-FAS titrimetric method was added to the field studies to gain a better understanding of the nature of the oxidant residual at the power plants. Exhibit 19 presents the results of two studies that were conducted in the laboratory to compare this method with the amperometric method. Chlorine (sodium hypochlorite) was added to 4ℓ samples of San Francisco Bay water, and oxidant residuals were determined for a period of 40 min. A good correlation between FOR determined amperometrically and titration A of the DPD-FAS method was obtained. TOR determined by the amperometric method was equal to or slightly less than TOR calculated from the DPD-FAS results (titration A plus B). The discrepancy between amperometric and DPD TOR data appeared to result from the underestimation of combined chlorine residual by the amperometric method. Results of titration C indicated that most of the FOR was bromine residual.

Exhibit 20 summarizes the disappearance of oxidant residual observed for all the in-plant studies. Loss of residual is expressed as a percentage of the chlorine dose. The table does not include the decrease of oxidant residual because of dilution immediately after the condensers. The measured TOR at the outfalls has been doubled before the percentage loss was calculated for those plants where such a

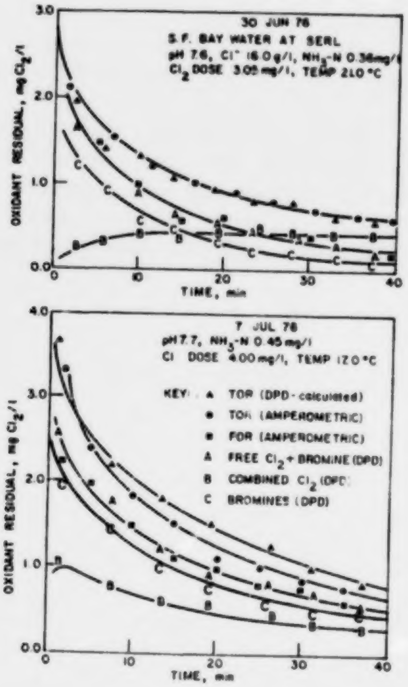


EXHIBIT 19: CHLORINATION STUDIES CONDUCTED AT SERL COMPARING THE DPD-FAS TITRIMETRIC AND AMPEROMETRIC TITRATION METHODS

EXHIBIT 20: SUMMARY OF LOSS OF OXIDANT RESIDUAL WITHIN THE POWER PLANT COOLING WATER SYSTEM

Power Plant	Unit	Approximate Flow Time (min)			TOR LOSS (%)*		TOR (%)* Remaining At Outfall†
		INJ. PT. To COND-IN	COND-IN To OUTFALL^b	Total	INJ. PT To COND-IN	COND-IN To Outfall†	
Contra Costa	6	1.2	1.5	2.7	55-75	8-21	17-24
	7	1.2	0.9	2.1	54-67	6-17	27-31
Hunters Point	4	1.5	0.9	2.4	44-59	10-34	24-40
Potrero	3	0.9	0.4	1.3	19-44	0-19	54-78
Moss Landing	6,7	0.8	6-7	7-8	10-32	41-64	16-27

* Percent loss does not include that caused by dilution immediately past the condensers.

† Sampling part at Moss Landing.

dilution occurred. Flow times between the sampling points at each plant are indicated. Results at Contra Costa are separated for Units 6 and 7 because of the different flow times from the COND-IN to the outfall.

Exhibit 20 shows that the disappearance of TOR generally depends on the flow time through the system. This is especially evident from the COND-IN to the outfall because of the wide variation of flow times that existed. Exhibit 21 is a graph of the percentage loss of TOR between the COND-IN and the outfall sampling point as a function of flow time.

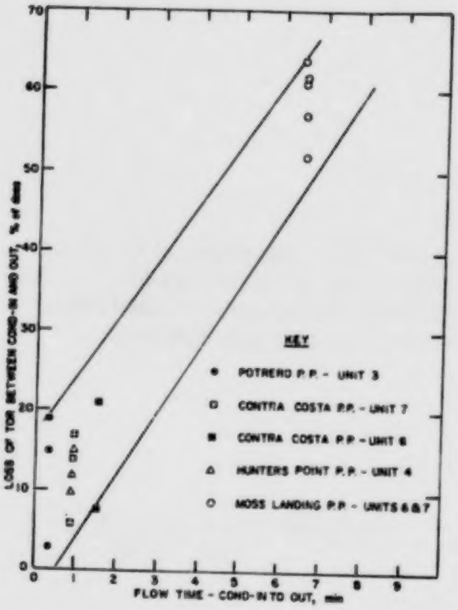


EXHIBIT 21: PERCENTAGE LOSS OF TOR BETWEEN THE COND-IN AND THE OUTFALL SAMPLING POINT

Decay and Receiving Water Studies

Decay studies were conducted on the chlorinated cooling water collected at the outfalls to determine the approximate life of oxidant residuals in the receiving waters. Oxidant residuals were measured at various locations in the receiving waters to determine the extent of the area affected by the power plant discharge during chlorination.

The results of the decay studies conducted at the outfalls are presented in Exhibits 22-25. The loss of oxidant residual between the point of chlorine injection, the COND-IN, and the outfall are shown. The effect of the 1:1 dilution, which occurred between the COND-IN and the outfall sampling point, is indicated by a dotted line for all plants

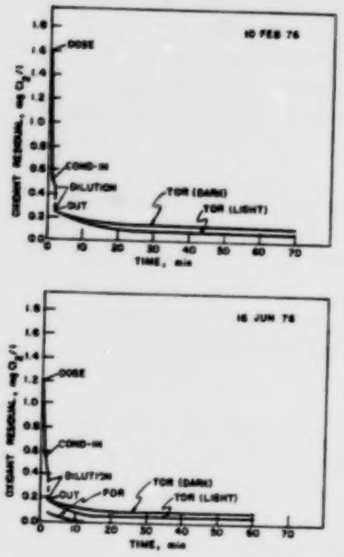


EXHIBIT 22: RESULTS OF DECAY STUDIES CONDUCTED AT THE CONTRA COSTA POWER PLANT, UNITS 6 AND 7 OUTFALL

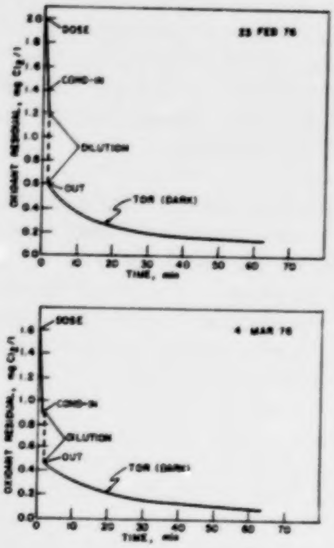


EXHIBIT 24: RESULTS OF DECAY STUDIES CONDUCTED AT THE POTRERO POWER PLANT, UNIT 3 OUTFALL

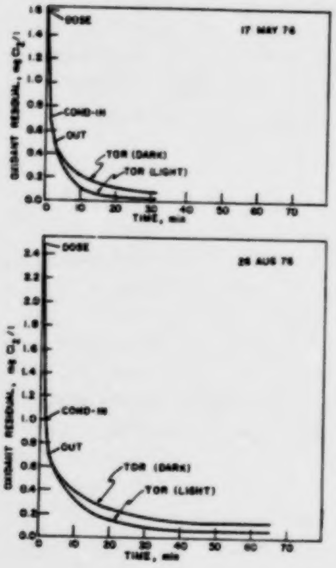


EXHIBIT 23: RESULTS OF DECAY STUDIES CONDUCTED AT THE HUNTERS POINT POWER PLANT, UNIT 4 OUTFALL

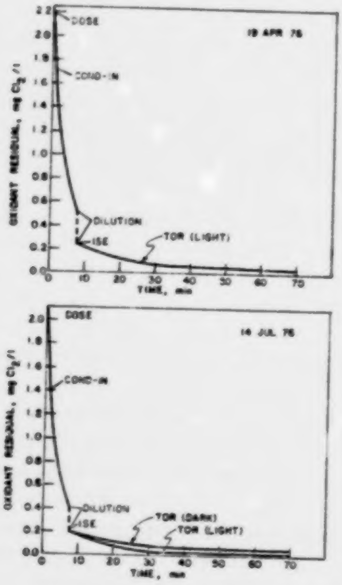


EXHIBIT 25: RESULTS OF DECAY STUDIES CONDUCTED AT THE MOSS LANDING POWER PLANT, UNITS 6 AND 7 AT SAMPLING POINT AT SHELL ENTERPRISES

except Hunters Point, where flows to both condensers were chlorinated simultaneously. Exhibit 26 is a summary of the decay studies. It should be remembered that the results are based on field studies, and that no attempt was made to keep temperature constant. In most cases, temperature increased in the "light" experiments and decreased in the "dark" experiments.

The results of receiving water studies are presented in Exhibits 27-31 in the form of isoconcentration lines of TOR at the surface. Surface TOR was always found to be the highest compared to samples taken at various depths. Whenever possible, the 0.02, 0.05, and 0.10 mg Cl_2/ℓ isoconcentration lines are shown together with the enclosed surface areas. These areas give an approximation of the regions affected by various levels of TOR.

The results of Contra Costa (Exhibit 27) show the effects of chlorine addition to one condenser of Unit 6. The study of 8 July 1976 was conducted during a flood tide so that no residual was detected downstream from the point where the discharge canal meets the river. The study of 3 December 1976 shows the effect of an ebb tide. A TOR of 0.02 mg Cl_2/ℓ was detected some 150 m (500 ft) down the San Joaquin River near the intake structure for Units 6 and 7. The plume boundary was clearly visible and was confirmed by both oxidant residual measurement and temperature readings.

Exhibit 28 presents chlorine addition to one condenser of Unit 4 at Hunters Point. A TOR of 0.02 mg Cl_2/ℓ was measured up to 150 m (500 ft) from the outfall structure. The dilution effect of varying tidal stages is evident. The studies of 19 October 1976 and 3 November 1976 were conducted at lower tides than the other two studies. Tidal effects are very pronounced because the discharge structure is submerged at high tides but not at low tides.

Results at Potrero are presented in Exhibits 29 and 30. Exhibit 29 shows the effects of chlorine addition to one condenser of Unit 3. A TOR of 0.02 mg Cl_2/ℓ was detected up to 168 m (550 ft) from the outfall structure and was drawn into the No. 1 and No. 2 unit intake. Tidal effects were not as evident as at Hunters Point because the Potrero outfall structure is never fully submerged. Exhibit 30 shows the effects of chlorine addition to all condensers of Units 1 and 2. These units are very old, and chlorination of each condenser separately is not possible. A TOR of 0.02 mg Cl_2/ℓ was detected up to 400 m (1300 ft) from the outfall structure.

A study was conducted in the receiving water at Moss Landing on 23 September 1976 (see Exhibit 31). It was very difficult to keep the boat in the discharge plume in Monterey Bay because of the force of 37.9 m^3/sec (600 000 gpm) of cooling water reaching the surface. It was difficult at times to determine the exact location of the plume because of waves continually moving the boat. A maximum TOR of

EXHIBIT 26: SUMMARY OF DECAY STUDIES

Date	Power Plant	Initial Temp. (°C)	Initial TOR ($mgCl_2/\ell$)	"Dark" or "Light"	TOR After				Time to Reach 0.1 $mgCl_2/\ell$ (min)
					30 min		60 min		
					$mgCl_2/\ell$	% of Initial TOR	$mgCl_2/\ell$	% of Initial TOR	
10 Feb 76	Contra Costa		0.23	Dark	0.14	61	0.12	52	> 90
				Light	0.12	52	0.09	39	45
16 Jun 76	Contra Costa	34.5	0.16	Dark	0.09	56	0.08	50	20
				Light	0.07	44	0.06	38	8
17 May 76	Hunters Point	24.0	0.52	Dark	0.10	17	--		30
				Light	0.03	6	--		10
26 Aug 76	Hunters Point	30.0	0.72	Dark	0.20	28	0.15	21	
				Light	0.12	17	0.08	11	38
23 Feb 76	Potrero	20.0	0.61	Dark	0.20	33	0.13	21	> 90
4 Mar 76	Potrero	20.0	0.43	Dark	0.15	35	0.10	23	60
19 Apr 76	Moss Landing	24.0	0.22	Light	0.08	35	0.03	13	22
14 Jul 76	Moss Landing	27.0	0.20	Dark	0.08	40	0.05	25	16
				Light	0.05	25	0.02	10	8

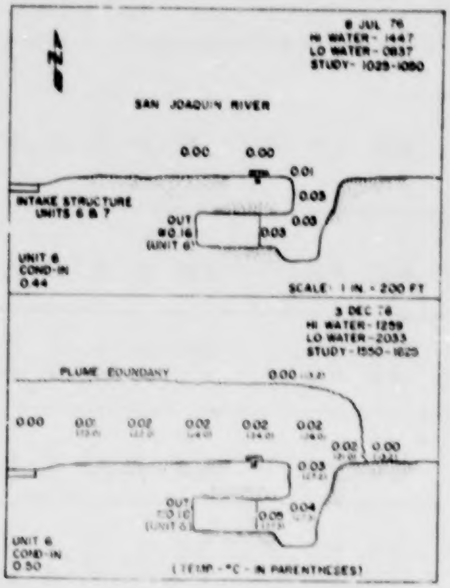


EXHIBIT 27: TOR (mgCl$_2$/ℓ) AT THE SURFACE OF THE RECEIVING WATER AT THE CONTRA COSTA POWER PLANT, UNITS 6 AND 7

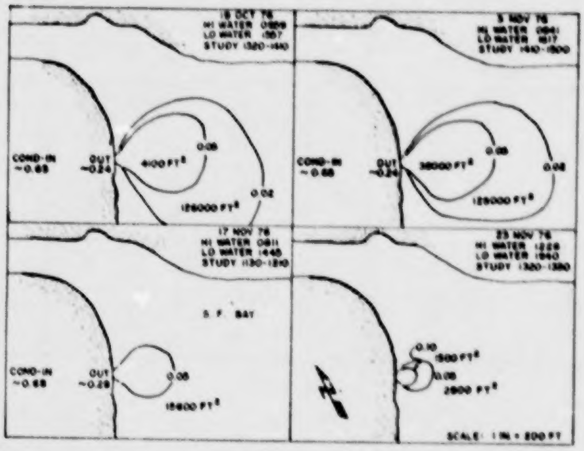


EXHIBIT 28: TOR (mgCl$_2$/ℓ) AT THE SURFACE OF THE RECEIVING WATER AT THE HUNTERS POINT POWER PLANT, UNIT 4

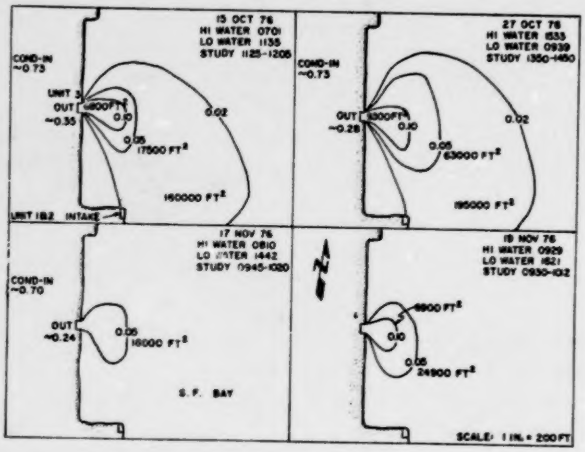


EXHIBIT 29: TOR ($mgCl_2/\ell$) AT THE SURFACE OF THE RECEIVING WATER AT THE POTRERO POWER PLANT, UNIT 3

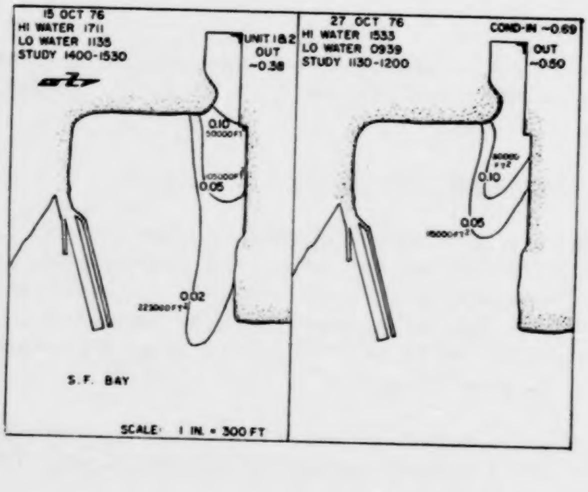


EXHIBIT 30: TOR ($mgCl_2/\ell$) AT THE SURFACE OF THE RECEIVING WATER AT THE POTRERO POWER PLANT, UNITS 1 AND 2

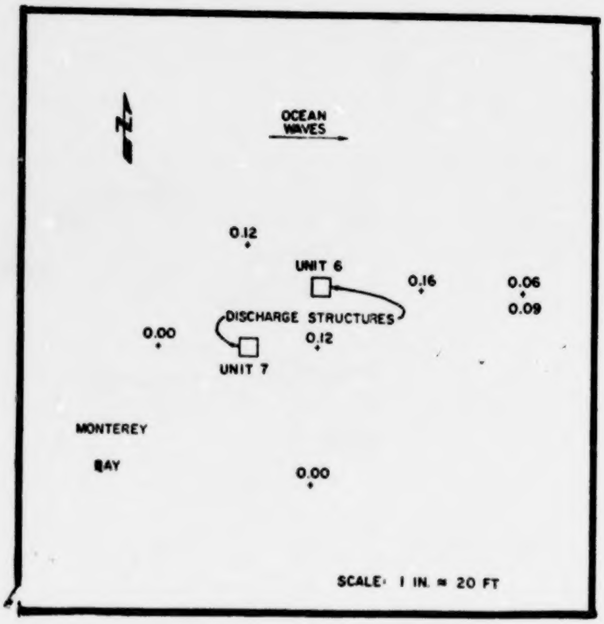


EXHIBIT 31: FOR ($mgCl_2/\ell$) AT THE SURFACE OF THE RECEIVING WATER AT THE MOSS LANDING POWER PLANT, UNIT 6

0.16 mg Cl_2/ℓ was determined at the surface at a time when the TOR ranged from 1.2 to 1.7 mg/ℓ at the COND-IN and from 0.31 to 0.33 mg/ℓ at the sampling point at International Shellfish Enterprises, Inc.

DISCUSSION OF RESULTS

In this section, we describe the nature of the oxidant residual, chlorination practices, demands in cooling water system, decay and receiving water studies.

Nature of the Oxidant Residual

The nature of the oxidant residual at each of the power plants is important for understanding the decay and persistence in the receiving waters. None of the methods currently available for the routine measurement of oxidant residuals unequivocally identify the compounds that compose the residual. This is especially true for oxidant residuals in estuarine and marine waters.

When chlorine is added to sea water that is free of amino-nitrogen, bromide (present at a concentration of approximately 70 mg/ℓ) is oxidized to bromine as follows:

$$HOCl + Br^- = HOBr + Cl^-.$$

Exhibit 32 shows the time required (based on reported rate constants) to convert 99% of the chlorine to bromine at a temperature of 25°C and a pH of 8.3 for varying percentages of sea water (1976). The reaction proceeds to 99% completion within 10 sec in high salinity water, but very little bromine is formed in water containing less than 3% sea water.

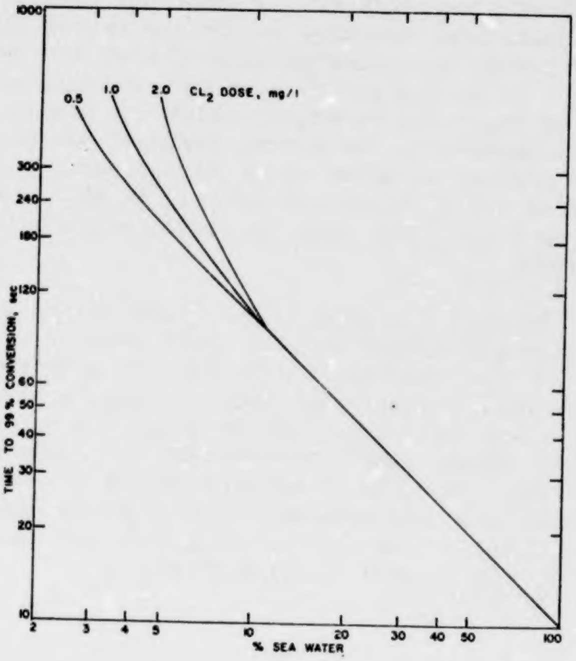


EXHIBIT 32: TIME REQUIRED FOR 99% CONVERSION OF FREE CHLORINE TO HOBr AT 20°C AND A pH OF 8.3

A similar oxidation of iodide occurs. Total oxidation to iodine would account for a 0.035 mg/ℓ residual as chlorine.

When ammonia-nitrogen is present in sea water, there is a competition between the ammonia and the bromide ion for chlorine. A number of reactions are possible, depending on pH, chlorine dose, concentration of NH_3-N, salinity, and reaction time. The formation of chloramines is generally favored by high NH_3-N, low salinity, high pH, and low chlorine dosage.

Using an equilibrium model, Sugam and Helz (1976) suggest that free bromine and bromamines predominate over the chlorine species at salinities above approximately 0.3 g/ℓ with ammonia concentrations "typical" of estuarine and marine waters. The predominant species in sea water containing 0.08 mg NH_3-N/ℓ dosed at 1.0 mg Cl_2/ℓ at pH 8.1 were tribromamine (NBr_3) and dibromamine ($NHBr_2$). Equal amounts of monochloramine (NH_2Cl) and HOBr were estimated to exist in a typical sea water of 35 g/ℓ salinity at pH 8 containing 0.12 mg/ℓ NH_3-N dosed at 0.5 mg Cl_2/ℓ. The model for 0.3 g/ℓ salinity is very complicated showing both chlorine and bromine compounds with no two species in abundance between pH 6 and 8.

Sollo et al. (1971) performed studies on the resulting speciation when solutions containing both ammonia and bromide were dosed with chlorine and residuals were determined after 2 min. Solutions containing 0.41 mg/ℓ NH_3-N and bromide concentrations of 3 and 25 mg/ℓ dosed at 2 mg Cl_2/ℓ are interesting, because they most closely model the conditions existing in cooling waters studied in this investigation. Very little free bromine existed over the entire pH range tested (4.3 to 9.0). At pH 7.4 and 25 mg/ℓ Br^-, a total halogen concentration of 2.8 mg Br_2/ℓ was detected, which was significantly lower than the 4.5 mg Br_2/ℓ expected. The entire residual was determined to be combined bromine and was believed to be $NHBr_2$, whose instability might explain the drop in total halogen residual. At pH 7.4 and 3 mg/ℓ Br^-, the entire 4.5 mg Br_2/ℓ total halogen residual was determined to be chlorine residual.

Exhibit 33, after Johnson and Overby (1971), shows the bromine species postulated to be present after 1 to 2 min as a function of both pH and the logarithm of the initial mole ratio of ammonia to bromine. These data suggest that HOBr and NBr_3 would predominate after 1 to 2 min under conditions that exist at the Hunters Point, Potrero, and Moss Landing power plants if a total conversion of chlorine to bromine is assumed. $NHBr_2$ would be predominant at somewhat higher ammonia concentrations than were measured at the power plants. At Contra Costa, both chlorine and bromine compounds are suggested with prediction of individual species being difficult.

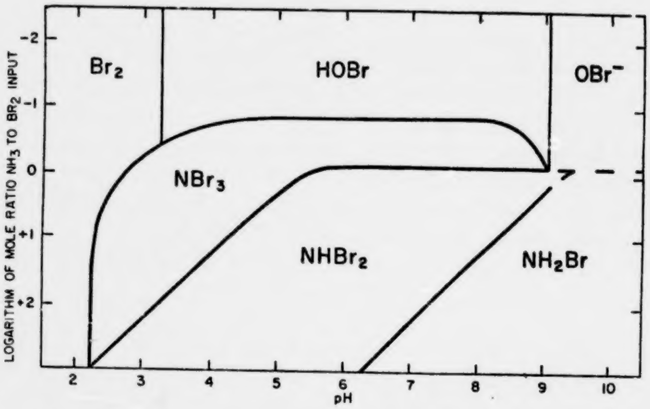


EXHIBIT 33: PRINCIPAL SPECIES OF BROMINE AND BROMAMINE PREDOMINATING AFTER 1-2 MIN AT VARIOUS pH AND AMMONIA TO BROMINE RATIOS. (Lines represent equal equivalent concentrations. Hypobromous acid separation from bromine given for 10^{-5} M bromide, after Johnson and Overby, 1971.)

It is apparent that both chlorine and bromine residuals generally exist when chlorine is added to estuarine and marine waters. Both the amperometric and DPD-FAS methods were used to determine oxidant residuals. The amperometric titration method for total residual determines all oxidant residuals whether they be chlorine, bromine, or iodine. It is apparent from Exhibit 19 that the amperometric free residual determination measures both free chlorine and bromine residual. Since the DPD-FAS method does not distinguish between free and combined bromine residual, it is possible that some combined bromine existed and was picked up by the amperometric method as "free residual." This has been suggested by Johannesson (1960), Palin (1961), and Sugam and Helz (1976). Therefore, the so-called "free chlorine" residual measured by the standard amperometric method in estuarine or marine water is neither totally "chlorine" nor totally "free."

Chlorination Practices

Chlorine dosage at many of the power plants was decreased during the course of this study. This was done both as a reaction to an awareness of greater than expected residuals and to decrease the residual in the discharge stream. Those plants using the orthotolidine method to set the chlorine dose were often chlorinating at twice the desired level because of the inaccuracies of the analytical method.

Total chlorine use per day for the four power plants located on the San Francisco Bay system averages about 0.30 metric tons/day (0.33 tons/day) based on 1971 figures (1973). This represents 1.25% of the total average daily use of chlorine of 34 metric tons/day (37.5 tons/day) for the 1975-1976 season as determined by Russell and Horne (1977).

Demands in Cooling Water System

Exhibits 20 and 21 indicate that the loss of oxidant residual in the cooling water system was a function of flow time. The longer the cooling water was held within the plant, the lower the oxidant residual that reached the receiving water. This loss of residual was a result of many factors, including reaction with ammonia-nitrogen, demand by inorganic reducing agents, reaction with organic nitrogen, and uptake by organisms living in the cooling water system. It is difficult to say which of these are most important. Exhibits 20 and 21 suggest the flow time through the cooling water system may be a good method to estimate the oxidant residual at various locations with varying doses of chlorine .

Decay and Receiving Water Studies

The decay studies (Exhibits 22-26) show that the most persistent residual existed at the Contra Costa power plant. From 50% to 52% of the 0.16 and 0.23 mg/ℓ TOR determined at the outfall remained after 60 min in those samples covered to exclude sunlight. From 38% to 59% of the 0.16 and 0.23 mg/ℓ TOR lasted for the same time period in samples exposed to sunlight. This persistence is likely explained by a slowly decaying combined chlorine residual that existed at Contra Costa.

The highest concentrations of TOR remaining after 30 and 60 min were at Hunters Point and Potrero. However, this was mostly a result of the greater initial concentrations of 0.43 and 0.72 mg/ℓ TOR. The lowest concentrations of TOR in decay studies were found at Moss Landing, where the long flow time to the outfall sample point produced small initial TOR of 0.22 and 0.20 mg Cl_2/ℓ. These were approximately the same initial TOR that existed at the Contra Costa outfall; however, the oxidant residual at Moss Landing decayed to much lower values. After 60 min at Moss Landing from 0.02 to 0.05 mg Cl_2/ℓ remained, while at Contra Costa from 0.06 to 0.12 mg/ℓ remained. The major difference between the oxidant residuals is the predominance of bromine residuals at Moss Landing, which decay more rapidly than chlorine residuals (Sollo et al., 1971; Johnson and Overly, 1971; Johannesson, 1960).

The presence of sunlight appeared to speed up the rate of decay at all plants. This effect was most noticeable during the initial 20 min decay at Contra Costa on 16 June 1976 when a FOR existed. After 20 min, only combined residuals existed, and the effect of sunlight on decay was less pronounced.

The significance of the oxidant residuals can be assessed in two ways. The first approach is to consider the effect on an organism such as a fish that is located at the discharge initially and then swims with a parcel of water away from the plume. The second method is to consider the effect on an organism that remains in one spot and is subjected to intermittent doses of chlorine. Such a case would be representative of benthos or of a fish that remained stationary in the discharge.

The first approach has been addressed by Mattice and Zittel (1976). Exhibit 34, which is derived from their data, shows several dose-exposure time combinations that result in acute toxicity; the chronic toxicity threshold is also indicated for marine and fresh water organisms. At Contra Costa, the cooling water discharge is into a canal that has an approximate residence time of 12 min. In the canal, average TOR concentrations were on the order of 0.05 mg Cl_2/ℓ. According to Mattice and Zittel, this combination of chlorine dose and exposure time would not result in acute toxicity to fresh water organisms. However, it is close to Mattice and Zittel's acutely toxic level for marine organisms. A TOR of 0.02 mg Cl_2/ℓ was detected for a distance of some 150 m (500 ft) down the San Joaquin River. Assuming an average water velocity of 0.55 m/sec (1.8 ft/sec), this results in an exposure time of 4.6 min at this TOR concentration. This TOR level is at Mattice and Zittel's chronic toxicity level for marine organisms. For fresh water organisms, the dose/exposure time stated to be acutely toxic to fresh water organisms by Mattice and Zittel is 0.02 mg/ℓ for 200 min.

EXHIBIT 34: ACUTE AND CHRONIC DOSES OF CHLORINE TO MARINE AND FRESH WATER ORGANISMS
(Mattice and Zittel, 1976)

Marine Organisms		Fresh Water Organisms	
TOR ($mgCl_2/\ell$)	Duration (min)	TOR ($mgCl_2/\ell$)	Duration (min)
0.20	0.50	0.10	25
0.15	1.0	0.05	60
0.10	2.5	0.02	200
0.05	13.0	0.01	550
0.02*	--	0.0015*	--

* Chronic toxicity threshold.

Current velocities in the discharge plumes have been determined by PG&E at the Hunters Point and Potrero Plants. At Hunters Point, the time to reach the 0.02 mg/ℓ TOR isoconcentration line is 45 sec. Assuming that the velocity is constant between the outfall and the 0.02 mg/ℓ TOR isoconcentration line, the estimated time to reach 0.05 mg/ℓ TOR is between 11-28 sec; with the same assumption, the time to reach the 0.10 mg/ℓ TOR isoconcentration line is 7 sec. These dose/exposure times are far below the values quoted by Mattice and Zittel to be acutely toxic to both fresh water and marine organisms. Concentrations of TOR exist in the receiving water that are above the chronic toxicity threshold for both fresh water and marine organisms.

At Potrero, with an average discharge concentration of 0.3 mg/ℓ TOR, the time to the 0.02 mg/ℓ TOR isoconcentration line is approximately 3 min. Using the same assumptions as for Hunters Point, the time to the 0.1 mg/ℓ TOR isoconcentration line is estimated to be between 48 and 56 sec; from the 0.1 mg/ℓ TOR to the 0.05 mg/ℓ TOR isoconcentration line is between 20 and 65 sec. The exposure between the 0.3 mg/ℓ TOR discharge and the 0.1 mg/ℓ TOR isoconcentration line (assumed to be an average of 0.2 mg/ℓ TOR) is predicted to be acutely toxic to marine organisms. Acute toxicity is not predicted according to the Mattice and Zittel data beyond this area. Chronic toxicity to marine organisms is predicted to exist out to the 0.02 mg/ℓ isoconcentration line and considerably further for fresh water organisms.

The 0.16 mg Cl_2/ℓ maximum concentration measured at the surface of Monterey Bay at the outfall from the No. 6 and No. 7 units at Moss Landing could only have existed for a flow time of a few seconds from the discharge pipes located at a depth of 6.1 m (20 ft). At a distance of some 15.3 m (50 ft) from the discharge, a residual of 0.09 mg Cl_2/ℓ was detected. For this residual to be acutely toxic, according to Mattice and Zittel's criterion, an exposure time of some 2.5 min must exist. It is questionable whether such a contact time existed between the discharge structure and a point some 50 ft away on the surface. These chlorine levels are above those suggested by Mattice and Zittel as chronic toxicity threshold values for both fresh water and marine organisms.

The second approach has been investigated by Dickson et al. (1977), who studied the effects of intermittent chlorination on goldfish and on protozoans obtained from attached growths. LC_{50} values for goldfish were found to be a function of total exposure time during a 24 hr period. In the temperature range of 17-22.5°C, 2 hr exposure/day resulted in an LC_{50} of 1.18 mg/ℓ TOR; with 3 hr exposure the LC_{50} was 0.71 mg/ℓ TOR; and with 4 hr/day exposure the LC_{50} fell to 0.63 mg/ℓ TOR. These authors concluded that two to three exposures of 15-30 min/day at a TOR of 0.5-0.75 mg/ℓ would not result in lethality to the goldfish. Concentrations in the ranges stated above were never detected in the receiving waters at any location sampled during this study. Indeed, these levels were detected in outfalls only in early studies at Hunters Point and Potrero before the reduction in chlorine dosage by Pacific Gas and Electric. Therefore, it might be concluded that with current chlorination practice no lethality would result from chlorine toxicity in cooling water to fish with the same response as goldfish.

The Dickson et al. (1977) analysis on chlorine toxicity to benthic protozoa was conducted at high (≥0.58 mg/ℓ) free chlorine concentrations over a short period (2 hr) and cannot be compared directly with the results obtained in this study. Suffice it to say that these workers noted significant decreases in numbers of individuals at all levels of free chlorine tested. Numbers of species and diversity were not affected below 1.45 mg/ℓ free chlorine dosed three times in 2 hr; there was a decrease in number of species when the dosing was increased to seven times in 2 hr at a level of 0.66 mg/ℓ. In general, it was noted that chlorine exposure generally caused a shift in population toward more tolerant species.

CONCLUSIONS

We itemize our conclusions below.

Chlorination Practice

1. Chlorination frequency varied among the five power plants studied from 1/week to 4/day. Duration of chlorination varied from 15 to 40 min per cycle.

2. Oxidant residual determined at the condenser inlets was the criterion used by the power plants to determine the level of chlorine dose.

3. During this study, the chlorine dose to produce the desired residual of approximately 0.5 $mgCl_2/\ell$ at the condenser inlet was reduced. This reduction resulted from a more precise measurement of chlorine residuals throughout the cooling system by our use of the amperometric method as compared to the orthotolidine method routinely utilized by power plant personnel.

4. All of the plants are currently using or are in the process of obtaining amperometric titrators because of the more stringent regulations that require accurate measurement of oxidant residuals.

In-Plant Studies

1. Total chlorine usage per day for the four power plants located on San Francisco Bay averaged about 0.3 metric tons/day (0.33 tons/day) based on 1971 figures. This represented 1.25% of the total average daily use of 34 metric tons/day (37.5 tons/day) estimated for 1975-1976.

2. Oxidant residuals determined at the condenser inlets were often significantly higher than the 0.5 or 1.0 mg/ℓ desired by plant personnel, especially at those plants that use the orthotolidine method for control of chlorine residual.

3. At Contra Costa, where the cooling water was the freshest, a combined residual of from 0.10 to 0.40 $mgCl_2/\ell$ existed from the condenser inlet to the outfall.

4. At Hunters Point, Potrero, and Moss Landing where cooling water ranged from 79% to 99% sea water, total oxidant residual generally equaled free oxidant residual at the condenser inlets. Some combined residual was detected at the outfall of Hunters Point and at a sample point 6-7 min of flow time from the condenser inlet at Moss Landing.

5. The DPD-FAS results indicated that most of the oxidant residual at Hunters Point and Moss Landing was bromine residual.

6. The disappearance of total oxidant residual through the cooling water system generally depended on the flow time. At Moss Landing, where the total flow time in the system was 6 to 7 min, from 16% to 27% of the oxidant input remained at the outfall. At Potrero, where the total flow time in the system was only 1.3 min, from 54% to 78% of the oxidant input remained at the outfall.

Decay and Receiving Water Studies

1. The slowest decay at the outfall was observed at Contra Costa, where 50% to 52% of the total oxidant residual of 0.18 - 0.23 mg/ℓ measured at the outfall remained after 60 min in these samples that were covered to exclude sunlight. For the samples exposed to sunlight, 38% to 39% remained after the same time period.

2. The most rapid decay at the outfall was observed at Hunters Point, where 17% to 28% of the total oxidant residual of 0.52 - 0.72 mg/ℓ measured at the outfall remained after 30 min in the samples covered to exclude sunlight. For the samples exposed to sunlight, only 6% to 17% remained after the same time period.

3. Maximum total oxidant residuals were found to exist at the surface of receiving waters.

4. During the chlorination cycle at Contra Costa, a 0.02 mgCl_2/ℓ residual was measured some 500 ft from the point where the discharge canal meets the San Joaquin River.

5. During the chlorination cycle at Hunters Point, 0.02 mgCl_2/ℓ residual was measured up to 500 ft from the No. 4 outfall and at up to 550 ft away from the No. 3 outfall at Potrero, and at up to 1300 ft from the No. 1 and No. 2 outfall at Potrero.

6. A maximum residual of 0.16 mgCl_2/ℓ was measured at the surface of Monterey Bay above the outfall from the No. 6 and No. 7 units at Moss Landing.

7. Using the approach of Mattice and Zittel, it was determined that all effluents produced receiving water oxidant residual levels that would be predicted to demonstrate chronic toxicity to marine organisms; the receiving water from two plants (Hunters Point and Moss Landing) showed levels that would be predicted to demonstrate chronic toxicity to fresh water organisms. Acutely toxic levels to fresh water and marine organisms existed in the receiving waters at the Potrero site.

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DISCUSSION

Dr. Riley: What kind of percent error do you get when you use the ampiometric titration method? What is the percent deviation for your performance of triplicate analyses of the same sample?

Mr. Hergott: We could not perform triplicate samples during the power plant monitoring because the level that comes through at the condensor is always varying.

Dr. Riley: Are you really positive that you have a two-fold difference there in your comparison?

Dr. Hergott: We saw the two-fold difference with the DPD method and the ampiometric method.

Dr. Baumgartner: Did you have any trouble getting information on chlorine use from the power plant officials?

Mr. Hergott: They were generally helpful.

From the floor: Has the state of California shown any interest in changing their standards?

Mr. Hergott: I have not heard anything about that yet. They are considering an appeal by PG&E to increase the 0.0 level. PG&E performed substantial work and presented data trying to change this; in fact, we obtained the data on receiving water studies from this work.

THE NBS PROGRAM FOR STANDARDS FOR TRACE ORGANIC ANALYSIS IN THE MARINE ENVIRONMENT

H.S. Hertz, S.N. Chesler, W.E. May, S.A. Wise, L.R. Hilpert,
J. Brown, A. Fatiadi, and F. Guenther*

The National Bureau of Standards (NBS) is currently conducting a research program to develop standards for trace organic analysis in the marine environment. In this paper, we summarize the results of these analyses for petroleum-in-sediment and petroleum-in-biota reference materials, marker compounds, and concentration techniques for polar organic materials.

PETROLEUM-IN-SEDIMENT REFERENCE MATERIALS

As previously reported to Environmental Protection Agency (EPA), a limited interlaboratory sample split was conducted to determine the suitability of sediment (∿1 ppm hydrocarbon burden) from the Katalla River, Alaska, as a petroleum-in-sediment intercalibration material. The results of this sample split encouraged the initiation of a more extensive intercalibration exercise.

An NBS sampling party collected ∿100 kg of intertidal sediment from the Katalla River area and from a relatively pristine Alaskan region, Hinchinbrook Island. The samples from each site were homogenized by mixing in a clean cement mixer for 3 hr, and samples of ∿300 g each were removed from the rotating mixer and placed in bottles. (Except for this brief period of homogenization, the samples were frozen from the time of collection.) Two bottles of sediment from each site were sent to 12 laboratories for hydrocarbon analysis by the laboratories' respective methodologies. The participating laboratories include four National Oceanographic and Atmospheric Administration (NOAA) contract laboratories (NOAA is partially funding the study), two EPA laboratories, two Bureau of Land Management contract laboratories, and academic, industrial, and private laboratories.

Samples were analyzed at NBS by headspace-sampling gas chromatography (GC), gas chromatography and mass spectroscopy (GC-MS), and

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liquid chromatography (LC) techniques. Since all the other laboratories use some type of solvent extraction to remove the hydrocarbons from the sediment matrix, samples were analyzed by an extraction method for comparison. The comparison of headspace-sampled and solvent-extraction values will determine if a bias exists for particular types of compounds between the two methods.

For this intercalibration exercise, the following data were compared:

1. Total hydrocarbons in GC elution range (approximately C_{10} - C_{30})
2. Total extractable hydrocarbons
3. Pristane/phytane ratio and the amount of each present
4. Percent water
5. Identities and amounts of the three most abundant aliphatic and three most abundant aromatic hydrocarbons
6. Total polynuclear aromatic hydrocarbon (PAH) concentration (four rings and larger)
7. Identity and amount of the most abundant PAH (four rings or larger).

The comparison of these data from the participants should provide a measure of the state of the art in trace hydrocarbon analysis.

Since some of the participating laboratories have not yet responded, the results of this intercalibration exercise will be included in the next report to EPA.

PETROLEUM-IN-BIOTA REFERENCE MATERIALS

The ultimate goal of this project is to release a biological trace organic standard reference material. However, immediate efforts have been directed toward the preparation and release of homogeneous materials contaminated with low levels of petroleum.

In conjunction with this effort, samples of Elizabeth River ("dirty") and Middle Marsh ("clean") oysters were obtained from Dr. Robert Huggett of Virginia Institute of Marine Sciences and Dr. F. Cross of National Marine Fisheries Services, respectively. Both sets of oysters were analyzed by a headspace-analysis GC procedure (see NBS Tech. Note 889) and by a modified procedure involving a high pressure liquid chromatography (HPLC) clean-up of the TENAX column prior to GC analysis. Results of these analyses are shown in Exhibit 1.

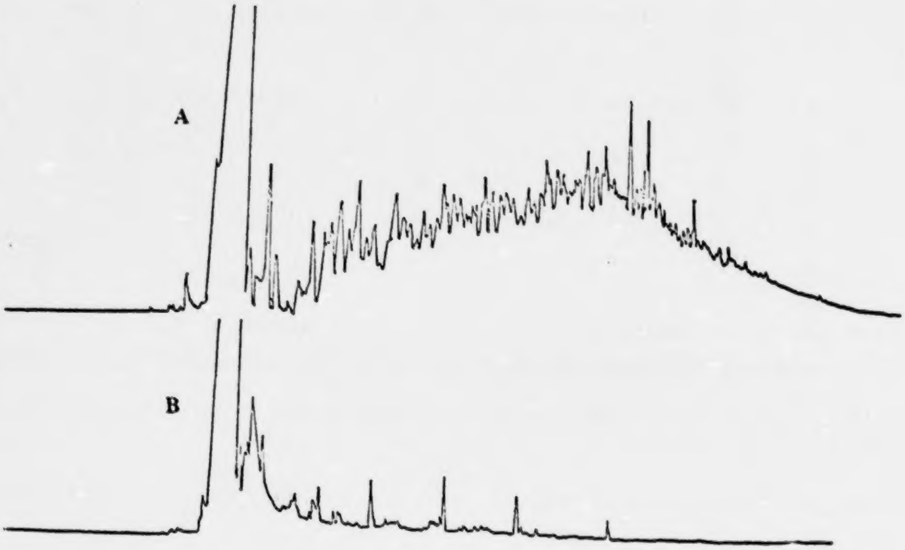

EXHIBIT 1: GAS CHROMATOGRAMS OF ELIZABETH RIVER (A) AND MIDDLE MARSH (B) OYSTERS

After headspace analysis, the residues left in each flask were extracted with n-pentane. The extracts were reduced in volume and analyzed for polynuclear aromatic hydrocarbon (PAH) content by HPLC, using ultravoilet and fluorescence detection. Exhibits 2 and 3 show LC elution profiles of the two oyster samples. The total PAH content, calculated in terms of 254 nm chrysene equivalents (i.e., assuming an absorptivity equivalent to that of chrysene at 254 nm), was 0.5 mg/kg for the Elizabeth River oysters and 0.05 mg/kg for the Middle Marsh oysters. Two of the individual PAHs (chrysene and 3,4 benzopyrene) in the Elizabeth River oysters were identified on the basis of retention volume and fluorescence emission spectra (see Exhibit 2).

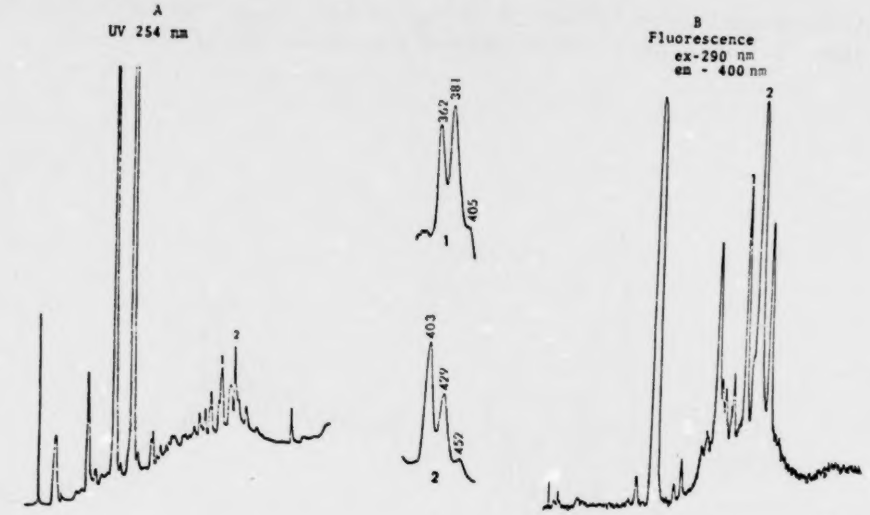

EXHIBIT 2: HPLC ELUTION PROFILES OF ELIZABETH RIVER OYSTERS

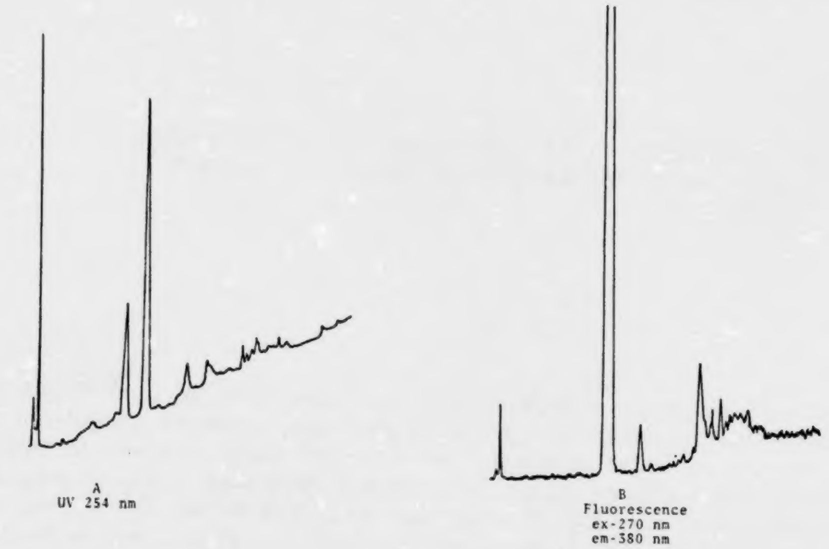

EXHIBIT 3: HPLC ELUTION PROFILES OF MIDDLE MARSH OYSTERS

MARKER COMPOUNDS

In this section, we describe marker compounds used as internal standards and used for the qualitative identification of petroleum.

Marker Compounds Used As Internal Standards

The height and area of chromatographic peaks are affected not only by sample size, but also by factors that influence the sensitivity of response of the detector, such as the flame ionization detector and the flame geometry, on column interactions or the molecular structure of the solute. If quantitative results are to be obtained, these variations must be carefully controlled. The effect of a variation must be eliminated by an appropriate compensation technique, such as the internal standard method.

Most of these variables can be nullified by referring peak height or peak area ratios to an internal standard. Use of an internal standard compensates for minor variations in column operation or detector response because both the internal standard and the sample are influenced to essentially the same extent. The concentration of any component can be calculated, using the following equation:

$$C_c = \frac{K_{is} C_{is} R_c}{R_{is} K_c}$$

where:

K_{is} and K_c = detector response factors

R_{is} and R_c = measured detector signals of the external standard and the sample component, respectively

C_{is} = known concentration of the internal standard.

In many cases where high accuracy (<5% error) is not required or the measurement of response factors is impossible or impractical, these response factors are arbitrarily assigned a value of unity.

An ideal internal standard will meet the following criteria:

1. It should yield a completely resolved chromatographic elution profile.

2. It should have an elution time nearly equal to that of the compound being measured.

3. The ratio of its peak height or area to that of the compound being measured should be close to unity.

4. It should not be present in the original sample.

5. Chemically, it should act similarly to the compound being measured.

6. It should have a response factor equal to that of the compound being measured.

7. It should be in equilibrium with the matrix that contains the compound being measured.

8. It should be added to the sample at the time of collection to compensate for losses of sample components.

The advantages of the internal standard technique are that sample size need not be known, and reasonable accuracy may be achieved without the exact reproduction of operating conditions from run to run.*

The requirements of an ideal internal standard are never realized in the analyses of real samples, with the possible exception of isotope dilution experiments. Therefore, certain compromises must be made when choosing an internal standard compound. The simultaneous use of more than one internal standard is a viable method that we have used for trace-level petroleum analyses.

Internal standards can be used to relate separate analyses on an absolute basis. For example, analyses of an environmental sample from an oil spill and of unweathered spill oil can be corrected for differences in extraction efficiencies through use of the same internal standard. Representative chromatograms can thus be directly compared, and the weight % of oil in the environmental sample can be calculated, using the following equation:

$$\frac{\sum_{n=a}^{b} h_n \cdot Wc \cdot hc_{is}}{\sum_{n=a}^{b} hc_n \cdot W \cdot h_{is}} \cdot 100 = \text{wt \% oil in sample}$$

where h_n = peak height of normal aliphatic hydrocarbon of carbon number $\underline{n}$ in sample chromatogram

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* Parts of this discussion of the interanl standard method were adapted from Dal Nogare, S. and R.S. Juvet, Jr., 1962. Gas-Liquid Chromatography: Theory and Practice, Interscience, New York.

hc_n = corresponding peak height from unweathered spill-oil sample chromatogram

h_{is} = peak height of internal standard in sample chromatogram

hc_{is} = corresponding internal standard peak height in unweathered spill-oil sample chromatogram

W = weight of sample analyzed

Wc = weight of unweathered spill-oil sample used as external standard.

Marker Compounds for Qualitative Identification of Petroleum

Seven samples were chromatographed on a 3.05 m (10 ft) x 2 mm glass GC column packed with 1% SE-30 coated on chromosorb WHP. The column effluent was split 1:1 into a flame ionization detector (FID) and a flame photometric detector (FPD). The FID is a universal organic detector; the FPD will respond only to compounds containing sulfur (or phosphorus, depending upon detector setting). Exhibits 4-10 are the dual chromatograms obtained (FID and sulfur FPD). The similarity in sulfur chromatograms between the Kuwait crude oil and the tanker spill oil is particularly notable. The spill oil was identified as a light Arabian crude oil, suggesting that sulfur fingerprints might be an easy method of identifying sources of oil pollution.

CONCENTRATION TECHNIQUES FOR POLAR ORGANIC COMPOUNDS

Initial liquid chromatographic separations of some nitrogen heterocycles and some phenolic compounds have been achieved, and a program in organic synthesis to prepare some of the larger hydroxylated aromatic compounds is underway. These compounds must be characterized before developing separations procedures for environmental samples.

Liquid Chromatographic Analyses

Liquid chromatographic methods have been developed for the analysis of two groups of polar compounds: phenols (including a naphthol) and N-heterocyclic compounds. These two groups are of current environmental interest because they are present in energy sources such as petroleum, synthetic oil, shale oil, and coal. Liquid chromatographic separations for both classes of compounds have been achieved, using both normal-phase and reversed-phase liquid chromatography.

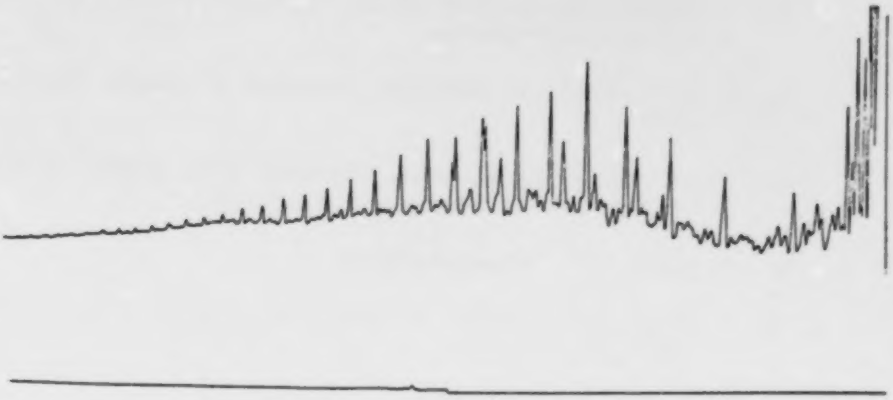

EXHIBIT 4: GAS CHROMATOGRAMS OF SOUTHERN LOUISIANA CRUDE OIL (Upper trace, flame ionization detection; lower trace, flame photometric detection)

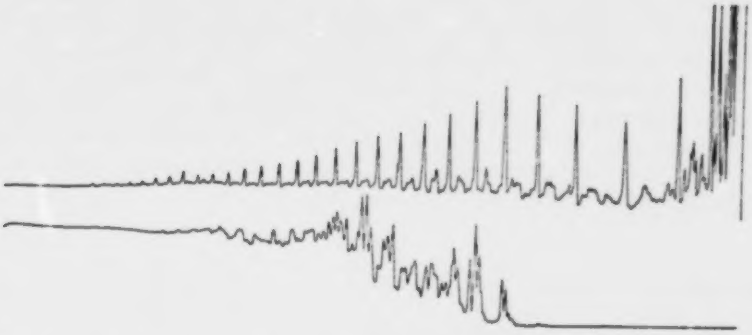

EXHIBIT 5: GAS CHROMATOGRAMS OF KUWAIT CRUDE OIL (Upper trace, flame ionization detection; lower trace, flame photometric detection)

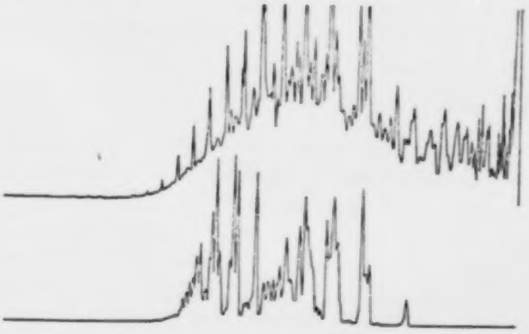

EXHIBIT 6: GAS CHROMATOGRAMS OF NO. 2 FUEL OIL (Upper trace, flame ionization detection; lower trace, flame photometric detection)

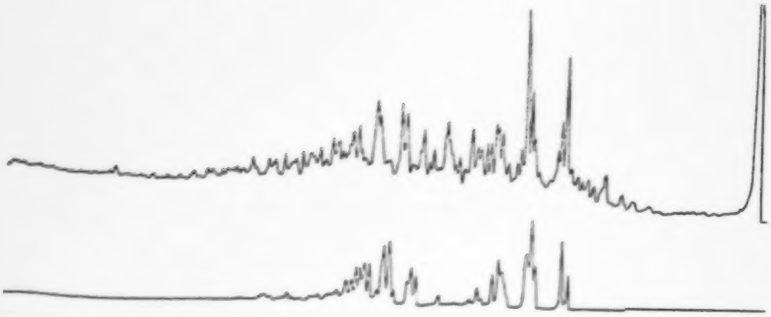

EXHIBIT 7: GAS CHROMATOGRAMS OF BUNKER C FUEL OIL (Upper trace, flame ionization detection; lower trace, flame photometric detection)

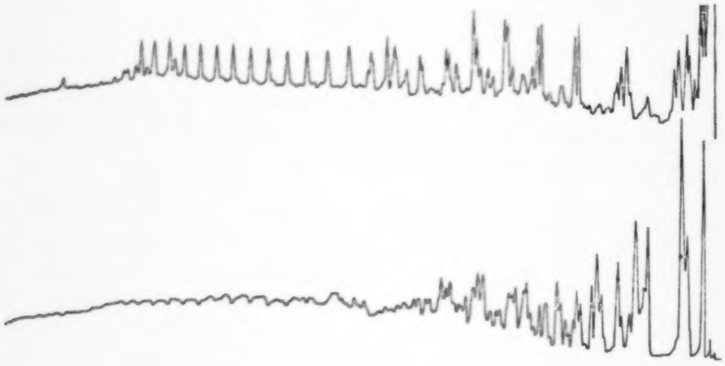

EXHIBIT 8: GAS CHROMATOGRAMS OF KUWAIT CRUDE OIL (Upper trace, flame ionization detection; lower trace, flame photometric detection)

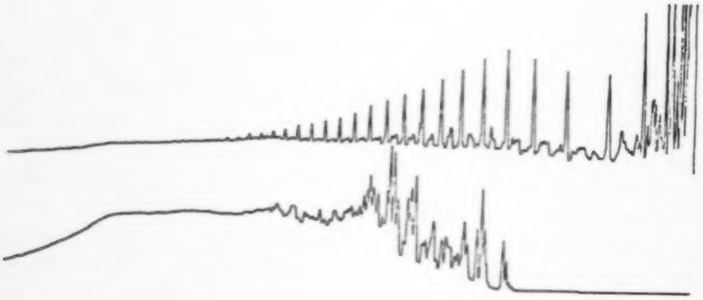

EXHIBIT 9: GAS CHROMATOGRAMS OF NO. 2 FUEL OIL (Upper trace, flame ionization detection; lower trace, flame photometric detection)

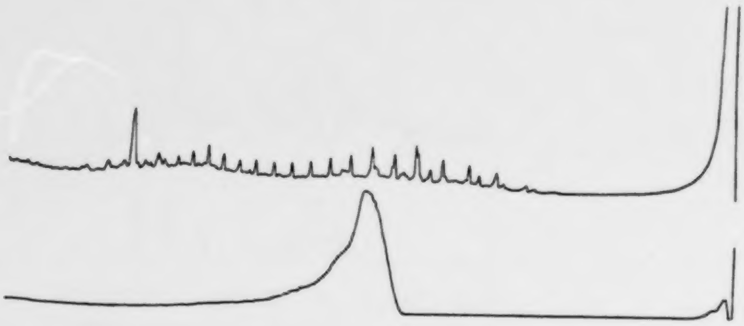

EXHIBIT 10: GAS CHROMATOGRAMS OF KATALLA RIVER SEDIMENT (Upper trace, flame ionization detection; lower trace, flame photometric detection)

The separation of a synthetic mixture of phenols using the reversed-phase mode (μ Bondapak C_{18}) and the normal phase mode (μ Bondapak NH_2) is illustrated in Exhibit 11.

Reversed-phase LC was also suitable for the analysis of a synthetic mixture of alkyl-substituted N-heterocyclic compounds, as shown in Exhibit 12. These compounds are representative of the types of N-heterocyclics commonly found in the same energy sources. This same mixture can also be separated on a μ Bondapak NH_2 column, using a nonpolar mobile phase as illustrated in Exhibit 12.

Synthesis of Hydroxylated Aromatics

The organic synthesis program started with the preparation of mono- and dihydroxyanthracenes. A review of the literature revealed several ways of synthesizing mono- and dihydroxy derivatives of polycyclic aromatic hydrocarbons. The first route involved careful sulfonation (fuming sulfuric acid) of a hydrocarbon. However, for the preparation of 1-anthrol, anthraquinone was sulfonated (in the presence of a mercury II - salt catalyst) to give an intermediate 5,10-anthraquinone-1-sulfonic acid (isolated as the K-salt). Subsequent reductive elimination (treatment with warm ammonium hydroxide and zinc dust) and hydrolysis (potassium hydroxide fusion) converted the intermediate into a somewhat air-sensitive 1-anthrol. A direct sulfonation of anthracene at 100-110°C produced anthracene-2-sulfonic acid, which was converted into relatively stable 2-anthrol. When the direct sulfonation was carried out at 150-170°C, the main product was anthracene-1,5-disulfonic acid; this was transformed by hydrolysis (i.e., fusion) into anthracene-1,5-diol. All compounds (except 1-anthrol) were purified by column chromatography [Florisil, 1:1 (v/v) ethyl acetate-benzene or silica gel, 1:1 (v/v) glacial acetic acid-benzene].

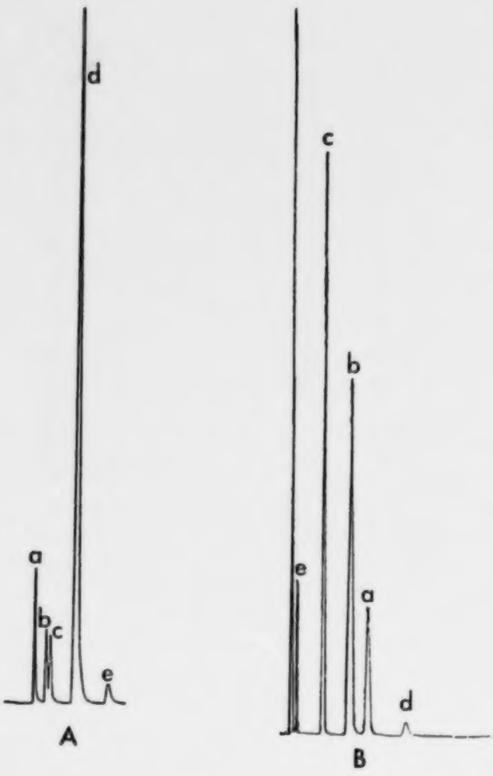

EXHIBIT 11: SEPARATION OF SYNTHETIC MIXTURE OF PHENOLS USING µ BONDAPAK C_{18} (A) WITH 40%/60% CH_3CN/H_2O AS THE MOBILE PHASE AND µ BONDAPAK NH_2 (B) AS 85%/15% CH_2CL_2/HEXANE AS THE MOBILE PHASE [(a) phenol, (b) p-cresol, (c) o-cresol, (d) 2-naphthol, (3) 2,4,6-trimethylphenol]

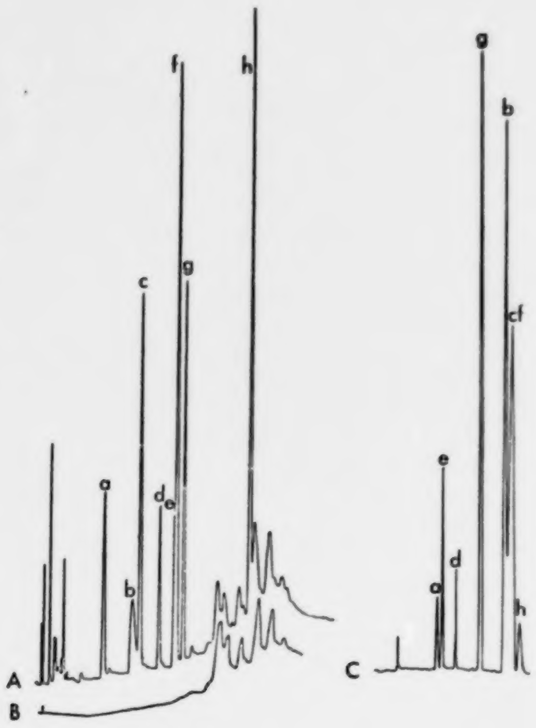

EXHIBIT 12: (A) SEPARATION OF N-HETEROCYCLIC MIXTURE ON µ BONDAPAK C_{18} WITH LINEAR GRADIENT OF 25-100% CH_3CN IN H_2O IN 40 MIN [(a) 2,5-dimethylpyrole, (b) 4-ethylpyridine, (c) lepidine, (d) 6-methyl 1,2,3,4-tetrahydroquinoline, (e) 2,3-dimethyldibenzocarbazole];
(B) BLANK GRADIENT FROM 25-100% CH_3CN IN H_2O IN 40 MIN;
(C) SEPARATION OF N-HETEROCYCLIC MIXTURE ON µ BONDAPAK NH_2 (with linear gradient of 0-35% CH_2CL_2 in pentane in 40 min at 2 ml/min) [(a) through (h) same as in (A)].

A second procedure for preparing hydroxylated aromatics involved osmium tetraoxide hydroxylation of a hydrocarbon (to give a cis-diol) and careful acid-catalyzed elimination to yield a mono-hydroxy product. Using this procedure, pyrene was hydroxylated and cis-4,5-pyrenediol was hydrolyzed to mono-hydroxy pyrene. This procedure was also applied in preparation of some unique polyhydroxylated aromatics (e.g., tetrahydroxyanthracene).

The third procedure for preparing hydroxylated aromatics involved oxidation (chromic or periodic acids) of polycyclic, aromatic hydrocarbons to quinones, which were converted either to trans-diols (via sodium borohydride reduction) or aromatic hydroxy-compounds (via sodium dithionate treatment). Using this procedure, phenanthrene, pyrene, triphenylene, and chrysene have been oxidized to quinones. These intermediates must now be converted to the hydroxy compounds.

The purity of all intermediates and new compounds prepared was verified by TLC, infrared, and mass spectrometry. Future work will include improvement in the procedures for sulfonation and fusion, improved osmium tetraoxide hydroxylation (including decomposition of the osmate ester), and acid hydrolysis in the aromatic cis- and trans-diols.

DISCUSSION

From the floor: Did you say what the results of your analyses were?

Dr. Hertz: We have the results from 7 of the 13 laboratories. The results varied according to the parameter reported. For example, while everybody agreed on water content within a few percent, the total extractables varied over a range of roughly 50 (which we considered reasonably good) at the low ppm or ppb levels.

From the floor: When do you expect to come out with your first standard, and what will that be?

Dr. Hertz: I would assume that the first standard might be something on the order of these water columns that I was discussing. Maybe in 2 years we could complete the stability test and various other studies for such a sample. We hope that this would be a certified standard reference material: one at which we had actually certified the levels with a certain level of confidence.

Dr. Riley: Have you decided what type of criteria you would place on the solubility of aromatic compounds in, say, distilled water? For example, have you done any kind of experiments in which you have actually filtered the water to which you have added the aromatic compounds to see if there are any changes in the concentrations?

Dr. Hertz: No. Using these so-called generator columns, we have pumped at various rates through the columns and have found that the concentration is essentially independent of the pumping rate. We have also subsampled as little as 100 mℓ and have found the concentration to be the same within this amount and the batch. I have not examined whether this is dispersed or solubilized material. We assume that it is dissolved.

Dr. Riley: When you do analyze these samples, do you just break the ampule open and then take a sample out?

Dr. Hertz: That is what we did in the initial case with the ampule material. Generally with the generator columns, we have been monitoring the effluent from the generator column directly in a UB detector or an LC system.

Dr. Dieter: I have been collecting data from petroleum hydrocarbons in different invertebrates. Have you published data on the concentration of these various substances in shellfish?

Dr. Hertz: No. We do have a manuscript describing the analytical technique that we have been using for the hydrocarbons in biota; in that, we give some data for various shellfish samples.

From the floor: How are you planning on homogenizing the tissues? Are you planning on freeze-drying them or wet freezing, or what?

Dr. Hertz: We will first study some middle samples, under the joint sponsorship of the EPA program and the NOAA/BLM program. At this point, we intend to thaw the samples, homogenize them in an ultrasonic probe in the presence of KOH, refreeze them, and release a digest, instead of freeze drying.

Dr. Richards: I would like to share with you some of the experiences that we have had at the Gulf Breeze Laboratory which sound very similar to the types of high-pressure liquid chromatography system that you have been using. We have found that it is very helpful to use appropriate elution gradients when working with the microbondapack C-18 column. I assume that you did use gradients.

Dr. Hertz: C-2 nitrile water gradient.

Dr. Richards: Exactly. By varying the type of gradient and the concentrations, we have been able to separate krysene and other things from very complex mixtures. We have also been using dimethylsulfoxide. If you do use dimethylsulfoxide gradients with this particular column (the C-18 microbondapack reverse phase), you must maintain a high, uniform temperature in the column to keep the flow rate appropriate. In that way, you can perform some separations that you cannot get with the aceto nitrile.

Dr. Hertz: We have not worked with dimethylsulfoxide. However, we can put a sample on a microbondapack NH2 column and do normal elution with pentane. By doing this, we can get a separation by ring size off the microbondapack NH2; we can separate aliphatics from the one ring and the alkalated one-ring compounds, which come off in one piece, then the two-ring, the three-ring, and four-ring, and so on. As a matter of fact, within the four-ring, we begin to get some separation of the different four-ring polymeric hydrocarbons off the microbondapack NH2; but again, always eluting in the same peak, to the alkalated and the non-alkalated parent of PNA. Those fractions can be put on a microbondapack CAT reverse-phase column to obtain beautiful profiles separating out. You can actually obtain flourescent submissions for identification. We can also take those fractions and put them on GC-MS.

Dr. Richards: Right. In field materials, we found a peak that is very similar to crysene. I just want to point out the hazard of finding peaks in the field that appear to be very similar to those others. In particular, we have been working with preparation of samples from lugworms, shellfish, fish, and algae.

You also mentioned the fluorescence spectrum work. We have had orders of magnitude of increased sensitivity using fluorescence. In addition, we have been working with Dr. Bens of the Coast Guard, who has a rapid scanning device that can be used to continually measure the fluorescent spectrum as it is eluted from the high-pressure liquid chromatogram.

Dr. Hertz: The identifications made on the basis of fluorescent emission spectra were all confirmed, at least to the extent of molecular weight by the GC-MS.

From the floor: You mentioned that you are 2 years away from a certified standard? What about an interim standard? Are you interested in working on the development of a standard that could be issued in a lot less time but without the quality of a certified standard?

Dr. Hertz: We are interested in something on the equivalent of research material. This is essentially what we are looking at in terms of the sediment and the biota. I do not know that we really want to get involved in water because we are very concerned about adsorption on containers, and so on. I do not know that we could even say something was homogeneous and hope that people using different methods could come up with the same answer on something that was delivered in a glass ampule.

Dr. Lefcort: I believe the question was, when could we expect to have research materials, interim standards?

Dr. Hertz: In terms of sediment, there are limited quantities of materials around now, through the auspices, mainly of the OCS work.

We have supplied samples to several EPA laboratories as well as several university laboratories. We have not given very large-scale sediment material much consideration yet, nor are we being asked for intercalibration.

THE NBS PROGRAM FOR STANDARDS FOR TRACE INORGANIC ANALYSIS IN THE MARINE ENVIRONMENT

I.L. Barnes, H.L. Rook, and T.J. Murphy*

In the past several years, a particular emphasis has been placed on new materials to be used in the assessment of environmental problems associated with the production of energy from a wide variety of possible sources. The Analytical Chemistry Division of the National Bureau of Standards (NBS) has been conducting a research program concerning the production of new standard reference materials and the determination of new materials that can be used as standards in the analysis of inorganic elements.

The paper focuses on the use of raw materials, products, and residues of coal conversion and oil shale processes for possible standard reference materials; the development of standards for monitoring water quality associated with energy production; and the development of a resin disk preconcentration method and an improved analytical capability based on neutron activation.

ANALYSIS OF RAW MATERIALS, PRODUCTS, AND RESIDUES OF COAL CONVERSION AND OIL SHALE PROCESSES

In this section, we describe ongoing research on standard reference materials for coal and coal fly ash, oil shale, and the evaluation of source materials.

Coal and Coal Fly Ash

Coal-SRM 1632, Coal, which is certified for trace element concentrations, has been sold out. Two coals of different trace element concentrations have been obtained to replace this standard. One is a western sub-bituminous coal, and the other is an eastern bituminous coal. In general, the eastern coal is about an order of magnitude higher in

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trace element concentrations than the western coal. Each of these materials has been ground and sieved. The -65 sieve fraction was taken for each standard reference material. After blending, nine samples were taken from each coal, and the homogeneity of each proposed standard reference material was determined by using neutron activation analysis to examine eight elements. The results of these determinations indicate that the western coal is homogeneous within the precision of measurement (±2%). The homogeneity study of the eastern coal is currently in process. Upon completion of this work, each proposed standard reference material will be packaged and samples taken for trace element determinations.

A study of the moisture content and drying of these coals has been completed. Although the western coal was found to have an extremely high moisture content (18%), both coals can be dried by vacuum drying for 24 hr at room temperature or by freeze-drying for the same time. All analyses for certification will be on a dry weight basis.

SRM 1633, Coal Fly Ash, is also out of stock and in need of replacement. A replacement material has been located and ordered. Preparation and certification of this material are of high priority and will begin upon receipt of the material.

Oil Shale Standard

Two candidate oil shale materials have been obtained. One is a 25 gal/t shale, and the other is a 35 gal/t shale. They have each been ground to two mesh sizes: -65 mesh and -200 mesh. The -65 mesh materials are intended as reference materials for the Fisher assay. The -200 mesh materials are intended to be trace element standard reference materials. A drying study on this material has been completed, and the samples surveyed for trace element profiles; however, further work has been delayed because of the recent de-emphasis of shale oil production.

Source Material for Candidate Standards for Coals and Oil Shale

Two representative coal and two representative oil shale materials were analyzed for trace element profile using a newly developed multielement procedure. The data were used to determine similarity of material to the expired coal SRM 1632 and to ensure that the trace levels between materials were not redundant. The results of this survey are given in Exhibits 1 and 2.

EXHIBIT 1: ELEMENTAL CONCENTRATIONS (μg/g UNLESS INDICATED) AND ESTIMATED UNCERTAINTIES ATTAINABLE

	Penn Coal		Colorado Coal		SRM 1632 Coal	SRM 1633 Fly Ash
Na (%)	0.089	<5%	0.22	<5%	0.040	0.34
K (%)	0.39	<5%	0.012	5-10%	0.27	1.6
Rb	30	<5%			20	120
Cs	2.2	<5%	0.052	10-15%	1.4	7.9
Sr	90	10-15%	120	5-10%	161	1400
Ba	140	5-10%	73	5-10%	330	3100
Br	56	<5%	0.76	5-10%	19	6.8
Sc	5.6	<5%	0.53	<5%	3.7	27
Cr	35	<5%	2.4	<5%	20.2	130
Fe (%)	1.1	<5%	0.20	<5%	0.87	6.4
Co	6.3	<5%	0.53	<5%	5.6	40
As	9.1	<5%	0.35	5-10%	5.9	62
Se	2.4	5-10%	0.79	5-10%	2.9	11
Sb	0.52	5-10%	0.12	5-10%	4.3	6.9
La	16	<5%	1.7	<5%	11.3	89
Ce	30	<5%	7.9	<5%	20.4	160
Sm	2.6	<5%	0.23	<5%	1.83	14
Cu	0.55	<5%	0.058	<5%	0.38	3.0
Yb	0.91	<5%	0.15	5-10%	0.70	4.9
Lu	0.19	5-10%	0.030	5-10%	0.14	1.0
Hf	1.7	<5%	0.22	<5%	0.95	7.8
Ta	0.34	5-10%	0.33	5-10%	0.21	1.5
Th	4.4	<5%	0.52	<5%	3.0	25

EXHIBIT 2: ELEMENTAL CONCENTRATIONS (μg/g UNLESS INDICATED) AND ESTIMATED UNCERTAINTIES ATTAINABLE

	Oil Shale		SRM 1632 Coal	SRM 1633 Fly Ash
Na (%)	1.6	<5%	0.040	0.34
K (%)	1.0	<5%	0.27	1.6
Rb	63	<5%	20	120
Cs	3.4	<5%	1.4	7.9
Ba	540	<5-10%	330	3100
Sc	4.6	<5%	3.7	27
Cr	32	<5%	20.2	130
Fe (%)	1.8	<5%	0.87	6.4
Co	9.0	<5%	5.6	40
As	64	<5%	5.9	62
Se	3.5	<5-10%	2.9	11
Sb	2.8	<5-10%	4.3	6.9
La	20	<5%	11.3	89
Ce	34	<5%	20.4	160
Sm	2.5	<5%	1.83	14
Eu	0.52	<5%	0.38	3.0
Yb	0.85	<5%	0.70	4.9
Lu	0.18	<5%	0.14	1.0
Hf	1.5	<5-10%	0.95	7.8
Ta	0.30	<10-15%	0.21	1.5
Th	4.7	<5%	3.0	25

DEVELOPMENT OF STANDARD REFERENCE MATERIALS FOR MONITORING WATER QUALITY

The purpose of this task is to initiate, develop, and evaluate potential new standard reference materials to assist in the effective analysis and monitoring of aqueous effluents and overall water quality associated with energy production. These needs are especially critical because of the extremely large quantities of water required for energy production. This task is being coordinated through the EPA/Environmental Monitoring and Support Laboratory.

Fresh Water Standard (SRM 1643)

SRM 1643 is a standard containing 22 trace elements in ultra-pure water in 0.5M nitric acid. Eighteen of the elements are at the low to mid ppb range and selected to approximate the concentration means in the 20 major water basins of the United States. The remaining four elements (i.e., sodium, potassium, calcium, and magnesium), which are in the low ppm range, act as typical matrix elements.

The preparation of this material was based on the experience gained during the preparation and certification of mercury in water (SRMs 1641-1642) and on the results of stability and container studies conducted jointly by the Analytical Spectrometry and Activation Analysis Sections. The results of this work indicated that a successful standard reference material could be prepared at the levels indicated. In addition, conventional polyethylene was indicated as a suitable low-cost container material for the standard reference material, provided the container was placed in a vapor barrier seal to prevent the loss of water vapor.

Two hundred and twenty liters of the standard reference material were prepared in one batch and then bottled in specially cleaned containers. The actual certification program was then initiated.

The elements present have each been analyzed by two or more of the following methods: neutron activation analysis, isotope dilution mass spectrometry, spark source isotope dilution mass spectrometry, atomic absorption spectrometry, graphite furnace emission spectrometry, zeeman atomic absorption spectrometry, inductively coupled plasma emission spectrometry, and polarography. The results have been very gratifying with only a few small exceptions. These are being resolved and should be completed in the near future.

Finally, mercury was added to the original sample but without much hope that it would be stable enough for certification. It now appears, however, that this may be possible and completed results are expected when the standard reference material is available for sale by March 1977.

Mercury in Water Standard

The original mercury in water standard has been completely sold-out. Currently, a new set is being prepared. Analytical work is scheduled to start in March with certification completed by July 1977.

Sea Water Standard

The possibility of preparing a sea water standard reference material is currently being explored. This work is expected to include explorations of such possibilities as issuance of a natural (sterilized) sample, a freeze-dried material to be reconstituted by the user, and/or a material representing the extraction of the essential trace elements.

Suitable large storage containers have been obtained and cleaned. These will be used to hold large (55 gal) working solutions for method development. Arrangements have been made to acquire, as needed, natural sea water of various salinities and various levels of natural contamination. The first material, a clean estuarine (half-sea) water (salinity values of 17-20), will be obtained in the near future. Work to date has concentrated on the development of analytical procedures for the heavy metals in sea water.

Oyster Tissue Standard

Since oysters are known to concentrate the trace metals present in their environment, they have been proposed for a standard reference material. Sufficient oysters for a standard reference material were cleaned, freeze-dried, ground, and sieved. Samples were taken for homogeneity testing by neutron activation analysis. These analyses showed that the material was inhomogeneous for a few elements. However, when the same oyster tissue was cryogenically ground (using liquid nitrogen), sieved, and blended, the material was found to be homogeneous.

Further work on this material was delayed because of the possibility that the International Atomic Energy Agency was going to distribute a similar oyster tissue reference material. However, they have abandoned this project since almost all of their material was used for analyses. Therefore, we plan to continue work on this proposed standard reference material.

Ocean Sediment Standard

An ocean sediment has been proposed as a standard reference material for trace elements. During the current fiscal year, we plan to locate and acquire sufficient material for a standard reference material. The material will be ground, sieved, blended, and sterilized, and its suitability as a standard reference material evaluated.

Improved Irradiation Procedures

Detection sensitivity for trace elements in water using neutron activation analysis is dependent on the irradiation procedures for the samples. Past procedures for the irradiation of water samples in the NBS research reactor were limited in sample volume to 1 mℓ sealed in quartz ampoules and to a maximum irradiation duration of 1 hr. This reactor safety limitation was due to the hydrogen gas pressure buildup from the radiolysis of water. Because of the limited quantity of water that could be irradiated, the sensitivity for many elements was much lower than normally expected for other matrix types.

To overcome this problem, procedures were developed for the preconcentration of water samples by freeze-drying 10 to 100 mℓ of the sample and irradiating the residue. Although this procedure did substantially improve the sensitivity for most elements, it was time consuming and had potential problems because of the pre-irradiation sample manipulations.

To overcome the problems with preconcentration of water samples, a new technique was developed, tested, and approved for the irradiation of large volumes of water. Polyethylene bags, made from 4 mil thickness, high-purity linear polyethylene, were fabricated to match the inside dimensions of the standard NBS irradiation "rabbits." The bags were precleaned, filled with 15 mℓ of water, sealed, and irradiated for periods of from 5 min to 4 hr in the high-flux pneumatic irradiation facility of the NBS reactor.

The hydrogen pressure was found to reach pressure equilibrium after 15 min. The hydrogen diffusion through the polyethylene film equaled the hydrogen production, and the bags maintained physical integrity for more than 4 hr.

This technique has been used for more than 6 months and has greatly facilitated the analytical procedure for the multitrace element water standard.

The Determination of Arsenic, Copper, and Antimony in Water

Investigative work has been completed on the accurate determination of the elements included in the multitrace element water standard. Using the new irradiation procedures, the elements silver (Ag), aluminum (Al), cobalt (Co), chromium (Cr), iron (Fe), molybdenum (Mo), manganese (Mn), strontium (Sr), vanadium (V), zinc (Zn), selenium (Se), and mercury (Hg) could be determined nondestructively using various irradiation, decay, and counting times. Copper (Cu), arsenic (As), and antimony (Sb) usually are not amenable to nondestructive activation analysis. The most useful product radionuclides are short-lived and suffer interference from the dominant ^{24}Na (sodium) activity of the sample. Thus, these three elements require chemical separations from the matrix activity. Published separation procedures by J. Costa *et al*. (1975),* for Cu, As, and Sb are capable of producing accurate results at the sub-ppm level of concentration. However, these procedures are highly dependent upon the pH of the extraction system and are not easily adaptable to multielement analyses.

Among possible radiochemical procedures and reagents for group separation of elements, inorganic ion exchanges have received increased interest because of the high selectivity that can be achieved. Furthermore, by separating elements in groups instead of individually, and utilizing the high resolution of semiconductor detectors, workers can achieve simple low-cost and rapid analyses.

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* "Determination of Purity Levels in Ultrapure Chemicals by Activation Analysis," 1974-1975 Yearly Report of Institute of Jozef Stefan, Ljublijana, Yugoslavia.

A chemical separation procedure was developed for the group separation of Cu, As, and Sb from water. The procedure used two chromatographic columns containing cuprous chloride and tin dioxide stacked in series. Following irradiation, samples were adjusted to a 6 molar concentration of $HClO_4$, and the resulting solutions were passed through the columns at a flow rate of 2 mℓ per minute.

The separation scheme was tested on some botanical standard reference materials, which had excellent certified values for Cu, As, and Sb. These results are listed in Exhibit 3.

EXHIBIT 3: A COMPARISON OF NEUTRON ACTIVATION ANALYSIS RESULTS TO OTHER INDEPENDENT METHODS

Method	Element	Spinach	Pine Needles	Tomato Leaves
Neutron Activation Analysis	Cu	10.80 ± 0.20	2.98 ± 0.16	11.1 ± 0.20
Atomic Absorption	Cu	12.70 ± 0.40	3.20 ± 0.40	11.5 ± 0.20
Spectrofluorimetry	Cu	11.50	2.90	10.6
Neutron Activation Analysis	As	0.120 ± 0.01	0.20 ± 0.02	0.23 ± 0.03
Spectrofluorimetry	As	0.170 ± 0.05	0.21 ± 0.04	0.29 ± 0.05
Neutron Activation Analysis	Sb	0.043 ± 0.004	0.22 ± 0.01	0.12 ± 0.03

Following procedure verification, this separation was used for certification analysis on the multitrace element standard reference material.

Certification Analyses for Trace Elements

The instrumental and separation procedures developed were used for certification analyses on 13 of the elements in the new trace element in the water standard reference material. Because of their short half-lives, Al, Mn, and V were determined individually. Na, K, Mo, and Sr were determined instrumentally as a group with moderate half-lives, and Co, Cr, Zn, and Se were determined instrumentally as a second group with long half-lives. Cu, As, and Hg were determined, using radiochemical separations to isolate them from the matrix ^{24}Na activity.

For certification, duplicate samples were taken from five randomly selected bottles and the replicate samples analyzed for all elements.

DEVELOPMENT OF A RESIN DISK PRECONCENTRATION METHOD

A number of workers have used a commercially available chelating resin (Chelex-100) to separate heavy metals from sea water by conventional column chromatography. This material seems to have a number of advantages over other types of resins in that it chelates heavy metals and does not strongly hold sodium, potassium, calcium, and magnesium (the common sea water matrix elements), in most analytical procedures. The column procedure, however, is inconvenient in that the elements must then be stripped from the column and prepared for analysis.

To eliminate these problems, we have developed a method in which a filter disk is manufactured and the chelating resin is incorporated within the disk material (not simply layered on the surface). These disks, using standard filtering procedures, permit the extraction of the heavy metals from as much as 1 ℓ of water with nearly 100% efficiency. Using radioactive forms of each element, both singly and in groups, we have examined the efficiency of this technique.

The advantages of the resin disk procedure are:

1. Multielement analyses may be performed

2. The sample may be easily preconcentrated up to a factor of 1000

3. The samples exhibit long-term stability without special handling

4. A wide variety of analytical methods may be used, including several that can be used directly on the disk.

For example, Exhibit 4 shows the x-ray fluorescence spectrum of an aqueous solution of 20 µg of a number of elements filtered through two successive disks, and Exhibit 5 shows the same for a sample of 10 mg of urban dust.* These spectra were obtained directly on the disk, the base material of which offers no analytical interferences. Exhibit 6 illustrates the linearity of typical analytical curves over a wide range.

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* Note: tungsten and titanium are contaminants in the disk introduced during manufacture. These will be eliminated in subsequent lots.

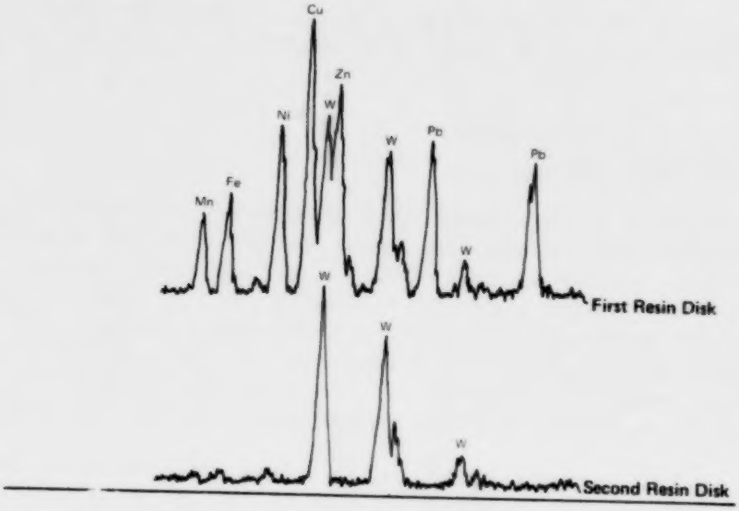

EXHIBIT 4: AQUEOUS SOLUTION: 20 μg EACH METAL FILTERED THROUGH SUCCESSIVE RESIN DISKS

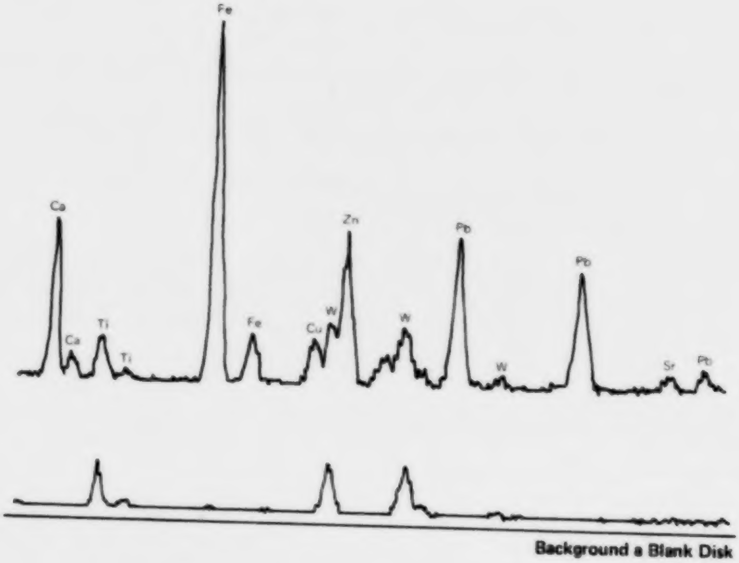

EXHIBIT 5: 10 mg ST. LOUIS DUST CHELATED TO A RESIN DISK

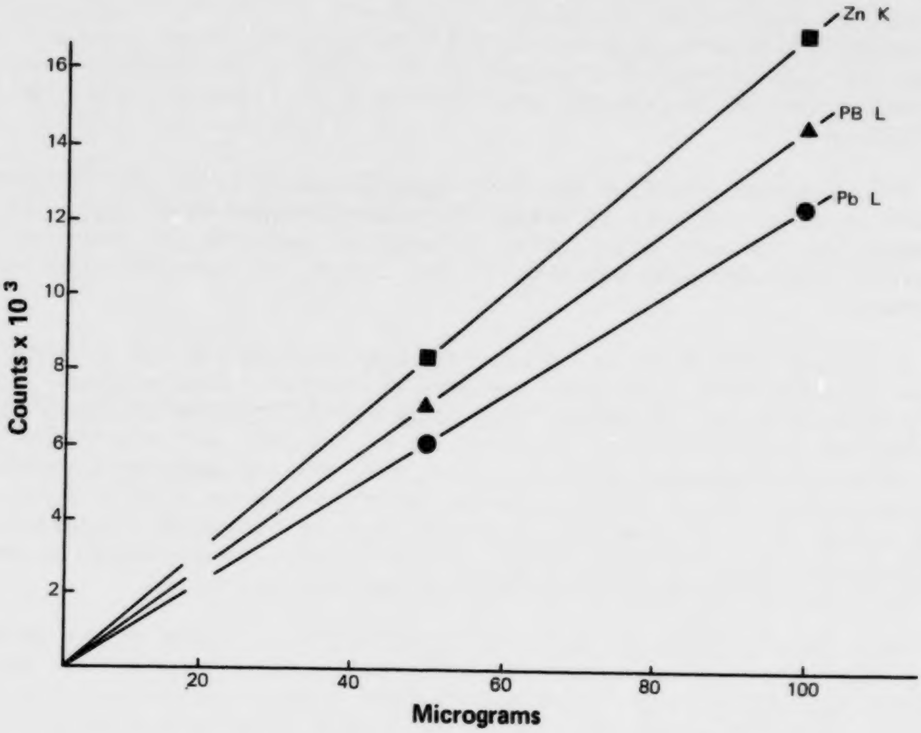

EXHIBIT 6: NONINTERFERING MULTIELEMENT ANALYSIS BY XRF OF RESIN DESKS

To date, we have used these disks for analysis by x-ray fluorescence, proton-induced x-ray (directly), and atomic absorption (by removing the elements from the disks). Activation analysis will be used in the near future.

DEVELOPMENT OF IMPROVED MULTIELEMENT ANALYTICAL CAPABILITY

A neutron activation system has been developed and evaluated for the accurate multielement analysis of diverse materials. This type of approach optimizes the analytical information obtained on a series of similar sample types while minimizing the manpower costs in the provision of this information. Therefore, this approach has potential applicability for more efficient certification analyses of environmental standard reference materials.

Radiochemistry is not always required for determining many elements in suitable matrices. In the most favorable cases, it is only necessary to irradiate samples and appropriate standards and to assay the resulting radioactivity with high-resolution semiconductor gamma detectors. Savings in time and effort, especially for routine analyses, are considerable, once the method has been developed to a desired level of reliability.

The equipment required for this type of analysis, in addition to the NBS reactor, consists of Ge(Li) detectors coupled with means for automatic and accurate positioning of samples, multichannel pulse-height analyzers with life-time and time-of-day clocks, and magnetic tape data recorders.

A crucial operation in any instrumental analysis is the reduction of raw spectral data into final analytical results. The analyst, in cooperation with his surrogate, the computer, is required to judge the qualitative and quantitative suitability of the data and select the best of several possible means for analyzing the information at hand. For our spectra, which usually contain 4,096 six-digit numbers from which dozens of peak areas and positions must be extracted, a large computer program is indispensable. Spectral data are processed at the NBS Computer Science Center by the computer code QLN1.

This program performs extensive qualitative analysis of the data to identify the peak characteristics of the isotopes sought. The quantitative comparison of the appropriate peak areas in samples and standards to arrive at elemental concentration and error estimates is performed for specified elements following qualitative analysis. This code is in a continual process of refinement, but the reproducibility and accuracy are excellent for well-resolved peaks and clearly superior to hand calculations. The analyst must still pay much attention to the quality of the input data; however, this essential feature of the instrumental analytical process is common to all methods of spectral data analysis.

A second feature of this system is the use of variable irradiation, decay, and counting times to maximize the numbers of elements obtained while minimizing the effects of interfering radionuclides. Optimum irradiation times, decay times, and counting times are selected on the basis of preliminary irradiation data and are applied to all other analyses on similar sample types.

The third and most essential feature of this system is that it has been extensively evaluated for accuracy using existing NBS standard reference materials as check samples. These samples have been analyzed in the multielement mode, and the results checked against the certified concentrations of the individual element in the standard reference material. The system now yields consistently good data on materials amenable to nondestructive neutron activation analysis.

DISCUSSION

Dr. Baumgartner: Was one of those early examples in fresh water?

Dr. Barnes: That was in fresh water, yes. However, we have since sampled sea water at low parts per million. We have used two different methods, by the way: activation analysis, which is the system of burning the material in a stream of oxygen; and an extraction technique.

From the floor: I am confused about the use of sodium, potassium, magnesium, and calcium matrix metals in the fresh water samples. Could you explain their use for a standard?

Dr. Barnes: These materials are interferences. If you are in a laboratory which is normally analysing water by biotonic absorption or by activation analysis, you would like a standard that is a close approximation of the same matrix material you meet in your everyday analysis; if we had left those out, we would have omitted a potential interference.

From the floor: What if you are running an analysis on materials that have a different matrix, for example, water samples that have high levels of hardness?

Dr. Barnes: If the material is there in this level, the addition of more to it does not create that much more of an interference. In other words, if you have a calcium interference, that is above this level, it does not make much difference how far it is above it, within reason. As long as it is there, interference will result.

Dr. Richards: I am concerned about the massive amounts of these salts that you are analysing on the filter from the sea water samples. You showed graphs with 20-100 mg of elements that naturally occur at 30 parts per trillion in that range. Does it work at those levels?

Dr. Barnes: Yes.

Dr. Richards: What are your flow rates?

Dr. Barnes: I have one here that has 80 mg of those four elements. I have the individual concentrations.

Dr. Richards: But none of them occur above 5 ppm in natural sea water?

Dr. Barnes: That is right. We have also done the same thing with natural sea water taken from the Virginia Institute of Marine Science. We are working right now on material that was gathered for us about 7 or 8 miles out, which should be much cleaner.

Dr. Richards: What are the approximate flow rates that you run through that filter? We do Kelex extraction work, using columns, and we typically run about 3-5 mills a minute.

Dr. Barnes: I am guessing that this is about 3 mills a minute, but at what pH do you do that?

Dr. Richards: We do normal sea water.

Dr. Barnes: Yes, and that will not work.

Dr. Richards: I disagree with you heartily, sir. It was published in ELANO, 2 years ago.

Dr. Barnes: You do not get nearly the same extraction efficiency as you do at a pH of about 5.2.

Dr. Richards: We do not modify our pH. We have done tracer experiments, to document that we do get complete recovery. It actually does work at those concentrations at 100 parts per trillion for cadmium.

Dr. Barnes: Yes. You can also work with Kelox-100. At a pH of 8.1 it acts as a healing ring. You can actually make Kelox act like a kelating agent; you can make it act like an ion exchange agent by changing the pH; and you can, in fact, get a mixture of these two by playing with the pH. We have done a great deal of work with this. Our tracer experiments show that it varies by how much sodium, potassium, calcium, and magnesium you are using, but you get a much better efficiency, if you go to a pH of about 5.2.

Dr. Richards: Do you have any plans to make a standard reference material of marine tissue?

Dr. Barnes: Yes. As you may or may not be aware, we first tried this with freeze-dried tuna fish, which did not work. Despite our best efforts, some of the bone was left in; the bone-seeking elements are not homogeneously distributed. The lead is reasonably well-distributed in this particular material. We have now prepared a sample of freeze-dried oysters. We initially had this material commercially prepared for us and it seems extremely homogeneous. We have found that, if we regrind the material, pyrogenically, under liquid nitrogen, it makes an extremely good standard. We will start certification, probably within a couple of months.

Dr. Richards: When you say "completely homogeneous," what sort of standard deviation would you get for something well-behaved, such as zinc or copper?

Dr. Barnes: At the Bureau of Standards, we try to analyze what people in the field actually need and anticipate what levels of precision they are going to need in the near future. We then try to certify it at somewhere between three to ten times better than that. The standard must be somewhat better than everyday use. For the well-behaved materials, we are looking at something below 1 percent. For some of the others, we will settle for about 1 or 2 percent.

STANDARDIZATION AND INTERCALIBRATION TECHNIQUES FOR MARINE MONITORING

Michael A. Basileo*

The objective of this project is to develop standardization and intercalibration techniques to assess the quality of marine environmental data. The tasks within the project are directed towards developing the capability to define the uncertainties or error boundaries associated with data obtained from environmental measurement systems. The project is composed of two tasks:

1. Standards development
2. Investigations.

STANDARDS DEVELOPMENT

The various standards under development within this task can be categorized by area of use, i.e., laboratory, interlaboratory or transfer, and field. In each case, the development is directed towards providing a product needed to define or reduce the uncertainty levels in a particular portion of the overall environmental measurement process. Thus, while the individual subtasks may appear unrelated, all support the project goal of providing improved definition of environmental data quality.

Laboratory standards are, obviously, used in the laboratory as the reference point for instrument testing and calibration. In most instances, these standards represent the laboratory's closest tie to national defining standards and, as such, represent the base of the overall measurement process, which culminates in field data. We describe below the seven subtasks: dissolved oxygen laboratory standard, dynamic test apparatus, environmental test specifications, conductivity/temperature/depth (CTD) transfer standard, pressure transfer standard, CTD field standard, and a handbook of underwater optical instrumentation.

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Dissolved Oxygen Laboratory Standard

Development of a laboratory dissolved oxygen standard is currently in progress. This standard will serve as a substitute for the Winkler method, which is currently used as the reference in the test and calibration of dissolved oxygen measurement systems. The standard will operate in a test bath over a range of -2^{o} to 35^{o} in temperature and 0 to 40 ppt in salinity. The dissolved oxygen range will span 0 to 15 ppm, with a measurement uncertainty goal of 0.03 ppm.

The Winkler method, which is a titration process, has demonstrated shortcomings that have led to this effort. Simplified, the measurement process consists of sample withdrawal, sample preservation, and titration. Each of these steps is extremely operator-sensitive; thus, it is difficult to obtain repeatable data within a laboratory and extremely difficult to obtain satisfactory agreement between different laboratories performing the same measurements.

The approach in developing the dissolved oxygen standard has been to reduce the degree of operator intervention in the measurement process. The standard will directly monitor oxygen concentration in the test bath by use of a submerged sensor. The sensor is similar to the polarographic membrane sensors presently utilized in field instruments. The measurement principle, however, has been modified to eliminate the errors caused by the membrane transport properties.

The dissolved oxygen standard is being developed under a contract with the University of South Florida. The first phase of the development will result in a prototype sensor, which will undergo testing at the test and evaluation laboratory in March 1977. Satisfactory performance of the prototype sensor will lead to completion of the final system and acceptance testing, which is scheduled for December 1977. The subsequent documentation of a proven alternative to the Winkler titration method is expected to encourage adoption of the standard by other laboratories and ultimately to improve the traceability of dissolved oxygen data.

Dynamic Test Apparatus

A dynamic test apparatus for laboratory testing of flow sensors is under development. The apparatus will generate controlled dynamics superimposed on steady flows of up to 70 cm/sec to establish flow sensor capabilities in the nonsteady flow environment. Present methods of testing water current sensors by determining their output over a range of steady-state speeds yield little information on the sensor performance under dynamic conditions. Dynamic conditions, however, represent real-world conditions in the marine environment when the sensor is subjected to motions induced by moorings, turbulence, and wave-generated water motions. Some initial efforts in the dynamic testing area have indicated that these errors can range from 20% to well over 100%. In attempting to assess the uncertainty levels associated with current measurements, it is necessary to have some knowledge of the dynamic response characteristics of the sensing system.

The Naval Ship Research and Development Center is designing and fabricating the test apparatus, which will be utilized on one of their tow carriages. The apparatus will be capable of two degrees of freedom and provide three modes of nearly sinusoidal dynamics: horizontal, vertical, and circular in a vertical plane. The peak-to-peak amplitudes can be varied from 0.4 to 1.2 m; the period is adjustable over the range of 2.5 to 12 sec. The apparatus can accommodate current sensors weighing up to 80 kg, to a maximum length of 1.8 m. Inducing these motions on the current sensor while the tow carriage is underway provides a simulation of steady current with superimposed fluctuations. To date, the vertical circular mode has been completed and tested. Hardware fabrication for the remaining two modes has been completed, and acceptance testing is to begin in March 1977. A final report documenting the overall dynamics simulation system will be completed in August. The apparatus can then be utilized in defining the errors in current measurement caused by dynamic effects.

Environmental Test Specifications

Environmental test specifications will be developed to provide a higher operational reliability for marine instrumentation. Presently, no standardized set of environmental test specifications exists that is directed toward marine instrumentation. Consequently, instrument designers do not have sufficient information about the environment in which their product is expected to operate. The usual result is underdesign rather than overdesign, a fact that has been documented by past testimonials on poor instrument performance. The solution to this problem lies in telling the instrument supplier what environmental conditions his product will encounter and having him demonstrate in a test laboratory that his product can survive these conditions with a high degree of certainty.

The environmental test specifications to be developed will provide the information on conditions encountered in typical marine-monitoring operations, which include shipping, shipboard storage and handling, temperature, humidity, shock, vibration, and salt spray. The specifications will be focused on the environment encountered in operations -- from typical marine research vessels ranging in size from the launch up to about 100 m in length. Future efforts will include buoys and other field environments in which instrumentation is used.

Work statement development will commence in the second half of fiscal year 1977 with a goal of awarding a contract by the end of the year. Completion of the contract is expected within 1 year after award. The resultant environmental test specifications would then be available for inclusion as part of an instrument procurement package and for use in performing the subsequent acceptance testing.

Conductivity/Temperature/Depth (CTD) System

Transfer standards are utilized to assess the comparability of results obtained between different laboratories performing similar calibrations. Calibration of environmental sensors is an area in which standardized procedures have not yet been fully developed. As a consequence, the calibration process tends to vary from one laboratory to another. Even when a common process is generally followed, variations in application tend to arise that have the potential of causing errors in the final result.

To define the uncertainty of a sensor calibration, which is a component of the overall field data uncertainty, it is necessary to evaluate the total calibration process. This can be accomplished by several laboratories calibrating the same transfer standard (i.e., a known, highly stable sensor) and comparing results to expected values. The "expected" values result from calibration performed at the "reference" laboratory. This designation can be applied to a laboratory based on its superior capability or can be applied arbitrarily to obtain a single point of reference. Results of the interlaboratory calibrations can be used to evaluate the effect of variations in calibration process and as a periodic check to verify that the laboratories in question remain in some constant relation to one another.

A conductivity/temperature/depth (CTD) system was selected as one of the transfer standard development areas. These three parameters are related to most environmental monitoring programs. The measurement goals of the transfer standard are 0.5 to 65 millisiemens/cm in conductivity with an uncertainty of ±5 microsiemens/cm; -3° to 30°C with a ± mK uncertainty; and 0 to 1500 decibars in pressure with a ± 0.75 decibar uncertainty.

An off-shelf CTD system was procured that showed the potential for having the high stability required in a transfer standard. Initial measurements have confirmed this stability. The system is presently undergoing a laboratory evaluation over a 6-month time interval to verify its measurement capabilities. In the summer of 1977, the system will be shipped to another laboratory for calibration. It will then be returned to the test and evaluation laboratory for final calibration and data analysis. The end result will be a report that defines the transfer standard capabilities and the procedures utilized for performing intercalibration. The measurement results obtained at the two laboratories will be presented as an example of a typical intercalibration experiment.

Pressure Transfer Standard

The development of transfer standards and intercalibration techniques for high-accuracy pressure sensors is currently in progress. These sensors, which are presently being employed in tide measuring systems, have an accuracy approaching that of the available standards. Piston gauge pressure standards operated with careful technique are marginally acceptable as calibration standards. Slight variations in the calibration process can generate errors that are several times larger than the allowable error of the calibration process. The capability to perform interlaboratory calibration will provide a means for detecting these variations, thus providing assurance that the calibration process remains within narrow boundaries.

Pressure sensors of the type utilized in the high-accuracy tide systems have been procured and will undergo a laboratory evaluation to define their characteristics. Pressure ranges of 0 to 10, 0 to 20, and 0 to 700 decibars were selected that correspond to the expected "worst case" measurement ranges in the laboratory calibration process. Upon completion of the laboratory evaluations, the sensors will be calibrated at another laboratory and then returned for final calibration and data analysis. The uncertainty goal for the interlaboratory comparison is ±0.01% of reading, which corresponds to the expected uncertainty of the laboratory standards utilized. The final report, to be completed in December 1977, will define the transfer standard capabilities and the intercalibration techniques, and will include the results of the intercalibration as an example.

CTD Field Standard

In the hierarchy of standards which shows the National Bureau of Standards at the apex, field standards would usually be shown at the lowest level. The purpose of the field standard is to provide some measure of assurance that the monitoring instrumentation has not changed characteristics during the course of a field operation. This capability would not normally be adequate to allow readjustment of a discovered shift; it would, however, serve as a flag indicating that the quality of subsequent data obtained with the instrument is suspect. For those experimenters fortunate enough to have spare monitoring instruments, detection of a shift would trigger instrument replacement.

A field verification standard for CTD sensing systems will be developed. The use of the CTD system is common to many monitoring activities; thus, the availability of a field standard for this system would provide improved definition of quality for a significant volume of marine environmental data. The verification system to be developed will provide an overall sensing system check. It would simulate an environmental parameter (e.g., a particular temperature), which should result in a certain system output within some defined tolerance level.

This development will begin in the second half of this fiscal year; plans are to award the development contract by the end of the fiscal year. The final product, expected within 1 year after contract award, would be the field verification system complete with the appropriate methodology for its use.

Handbook of Underwater Optical Instrumentation

The development of a users' guide for underwater optical instrumentation has been put into the field standard category, but it is actually an approach toward standardizing field methods. Many types of commercially available optical instruments are used in water quality measurement programs. Each type measures different optical properties, which can sometimes be correlated with other water quality parameters. Information on the theory behind the various measurements is available, but usually it is in a highly technical form which is not readily usable by the scientist, engineer, or program manager unfamiliar with optical theory.

The purpose of this handbook will be to describe the basic theory of operation of the various types of optical instruments, indicate the kinds of quantitative and qualitative information that can be derived from the provided measurements, and make recommendations on the best approach for specific applications.

A contract for the handbook was awarded to Scripps Visibility Laboratory in October 1976. Agreement has been reached on the final handbook outline; the handbook itself should be completed by January 1978. The completed text will be used to provide sufficient information to scientists, engineers, and program managers so that they can make an intelligent choice of optical instrumentation to meet the data requirements of their monitoring program. The text will also serve as a basis for the standardization of optical property measurement methods.

INVESTIGATIONS

In this task, we attempt to define the comparability of results obtained from different approaches to the measurement of chemical properties of marine waters. The trend has been to develop direct measurements (*in-situ*) systems to replace analytical laboratory methods in water analysis. The *in-situ* approach has several advantages; the most significant is probably cost per measurement. The accepted standards, however, are still defined in terms of analytical methods. To enjoy the benefits of *in-situ* monitoring without the loss of data quality, we must first demonstrate the traceability of the newer methods of the existing standards. The results of such demonstration then serve as a basis for defining the quality of data obtained from the *in-situ* measurements. This task includes the following subtasks: methods for salt water analysis, standards for *in-situ* chemical instrumentation, water quality test kits, and specific ion electrode evaluation.

Methods for Salt Water Analysis

The objective of this subtask is to determine the comparability between different methods for the chemical analysis of salt water. The Environmental Protection Agency (EPA) publishes standard methods for chemical analysis, most of which are applicable to both fresh and saline waters, in Methods for the Chemical Analyses of Water and Wastes (1974). The oceanographic community has no similar standard text, but one of the commonly accepted documents is A Manual of Seawater Analyses by J.D. Strickland and T.B. Parsons (1968).

In attempting to standardize a single set of analytical methods for saline water, it is necessary to determine whether conflict exists among the results obtained by the various available methods and to assess which of the methods provides the most reliable results in terms of data quality. The approach for this work has been to define 24 chemical parameters from four categories: nutrients, heavy metals, low molecular weight hydrocarbons, and other common analyses. The parameters were selected on the basis of those most certain to meet the present and future needs of the National Oceanographic and Atmospheric Administration (NOAA) and EPA in the area of sea water pollution monitoring.

Proposals have been requested that require the contractor to compare the analytical methodologies for the selected parameters using both open ocean and estuarine water. Contract award is expected in March 1977; completion of the contract and analysis of results are scheduled for September 1978. The final report will then serve as a basis for the recommendation for a standardized set of salt water analytical methods to be adopted by both NOAA and EPA.

Standards for In-Situ Chemical Instrumentation

An investigation is underway to assess whether there is measurement traceability between *in-situ* instruments and the corresponding analytical standards. The increasing availability of *in-situ* instrumentation for measurement of chemical parameters has resulted in significant change in operational practices. The analytical methods being replaced have been well organized in terms of documented methods and standardized procedures. Instrumental techniques are not yet as well organized; thus, the tie or traceability between the two measurement approaches remains largely unproven.

This study will attempt to fill the gap by determining the chemical parameters now being measured with *in-situ* instrumentation and the standards utilized to calibrate the instrumentation. These standards will be compared to those established for the analytical methods; based on the degree of comparison, the measurement uncertainties of the *in-situ* instrumentation will be estimated. Recommendations for changes in the existing standards for instrumentation will be provided where this would upgrade the comparability of the two methods.

A contract was awarded for the study in June 1976. A first draft of the report is expected in July 1977; the final report is due in September. The final report will provide guidance in developing the quality control practices required to assure the traceability of *in-situ* chemical measurement to accepted standards.

Water Quality Test Kits

An investigation has been initiated into the comparability between results obtained from standard analytical methods recommended by EPA and results obtained from the use of commercially available water quality "test kits." Inexpensive test kits for determining a variety of chemical parameters are available and used by many for water quality measurement. The use of these kits has been questioned by EPA and others, since little information is available on the quality of data they provide and their applicability under various naturally occurring background conditions. The chemical parameters under consideration for the comparison include ammonia, nitrate, dissolved oxygen, orthophosphate, and total alkalinity. Ten to fifteen specific kits will be selected by mutual agreement with the contractor. The tests will be run on samples in several backgrounds: fresh water, both clean and polluted; and sea water, low and high salinity, both clean and polluted. Contract award is expected by April 1977; completion of analysis and report is scheduled for March 1978. The report will provide guidance in the use and expected data uncertainties of water quality test kits for chemical analyses in natural waters.

Specific Ion Electrode Evaluation

An investigation is underway to determine the applicability of ion-selective electrodes to *in-situ* water quality measurements in natural waters. Many electrodes have been manufactured that are selective for various chemical species and provide simple, low-cost methods of analysis. Several of these ion-selective electrodes have been used to measure ions in natural waters.

There are, however, several problems with electrode measurement that have not been considered which jeopardize the quality of the data. All electrodes are subject to some type of interference, particularly in natural waters, and they do not respond directly to the total concentration in the sample but only to the free, "available" species. These stability and calibration errors have not been studied, nor have the response times, sensitivity to light, and flow variations been determined. There is much interest in using these electrodes as *in-situ* monitoring sensors, but their characteristics in natural waters are still undefined. Before ion-selective electrodes can be effectively used for *in-situ* applications, these problems and solutions must be defined to ensure that the level of data uncertainty in these applications can be predicted.

To define the effects of these potential sources of error, a series of evaluations of the ion probes have been initiated. Testing is already in progress on sodium, potassium, calcium, magnesium, and chloride electrodes from several manufacturers. Further tests will include fluoride and ammonia sensors and two probes for chloride and fluoride, which are currently in use on water quality monitoring systems. The testing, analysis, and report are scheduled for completion in October 1977. The report will present, for each class of electrode, results of the laboratory evaluations; thus, it will provide guidance in the use and performance of selective ion electrodes in in-situ applications.

DISCUSSION

Dr. McCammon: Are you going to recommend different manufacturers, then, and put a stamp of approval on different instruments?

Mr. Basileo: No.

Dr. McCammon: How are you going to separate out, say, the kits that will give reputable results and kits that will not?

Mr. Basileo: By presenting the test results of the experiment. We may end up with a particular kit with maybe three or four potential manufacturers. We will then present the results of the test in terms of how good they were, and it would be up to the purchaser. However, there should be enough data so we can tell that this one is clearly better than that one.

Dr. McCammon: I was particularly interested to see what you had been doing with the CTD. I know that the Navy has done an exhaustive study on CTDs to ascertain their reliability.

Mr. Basileo: When you say "Navy," our group used to be with Navy. Our work was spurred on by the fact that there are only one or two systems on the market that have the potential stability that would be usable for this application.

From the floor: Which instrument?

Mr. Basileo: We selected the Neal Brown, which is still being studied by the Navy. As a matter of fact, we are conducting the evaluation study for them, simultaneously assessing its use as a transfer standard for our purposes.

Mr. Stekoll: When you say that the Pacific ion electrodes have not been characterized, what do you mean? Don't the companies characterize them?

Mr. Basileo: Yes. Companies characterize most instruments and they publish the specifications. It has been our experience over the years, in testing instrumentation, that some 80% to 90% of instruments that come from the manufacturer do not meet the manufacturer's specifications. Another high percentage do not work when we get them.

From the floor: You said that your target is September 1978 for the development of standard analytical methods for nutrient heavy metals, molecular weight hydrocarbons, plus others. Would these be EPA/ NOAA standard procedures?

Mr. Basileo: Yes. We are comparing the oceanographer's method to the EPA's methods. We should end up with a good set of procedures for salt water.

From the floor: My reservations deal with how you are going to prove the accuracy of the methodology. Just about everyone does his own procedure in the oceanography field.

Mr. Basileo: In some of the areas, for example, heavy metals, there are no corresponding methodologies in an oceanographer's bible, so this is basically a comparison of two EPA methods.

From the floor: In the fresh water area, the American Public Health Association produces standard methods for water and waste water, and there are organizations such as ASTM, which is a standard-setting organization. Before they adopt a method as a standard, they do undertake rather rigorous testing procedures to determine its precision and accuracy. I question whether a contractor is capable in all these analyses, based on a comparison of one method to another, to derive standard procedures for EPA and NOAA.

Mr. Basileo: The outcome of this contract is going to be a comparison of methods: it will not automatically be a standard procedure. We are looking for a good set of procedures for ocean water.

Dr. Lefcort: There is a severe need for standardization for many of those parameters. If EPA/NOAA were to come out with a standards methods manual, it would be used internationally. However, unless there are some excellent precision and accuracy studies performed on each method and interlaboratory studies and round-robin testing procedures establish these variances, I did not want to see them come out as official procedures at this time. And I do not see what a comparison of two methods by one contractor is going to tell us. Where do we go from there?

Mr. Basileo: I agree that the output of this would certainly not be accepted by a lot of people until more work has been done on it. The outcome of this should give a set of methods to start with. Granted, they will not be accepted standards, but they should be a set of methods so that the detailed precision analysis, and the necessary intercalibration for developing the final, accepted standard method can be done.

Dr. Lefcort: Well, EPA-Cincinnati, has the Analytical Reference Service, which sends samples to approximately 80 laboratories. Based on their results, they calculate the precision and accuracy of the methods and publish it as an Analytical Reference Service Report, which, in turn, is adopted by other standards-setting organizations.

I believe that this is very necessary in the case of sea water analysis. The problem with the Cincinnati Laboratory is that they have not focused on sea water analysis and they are badly underfunded. The future of funding for this type of work does not look too promising.

Dr. Barnes: How can you possibly come up with a standard method when there are no standards? The second question is, how did you choose the EPA flame atomic absorption and graphite furnace methods as the potential methods to use. If you look at the data from those methods, which is in the EPA book, or the data which is published from all the laboratories, it is bad. There is no way you can use those methods.

DEVELOPMENT OF OCEAN OIL SPILL CONCENTRATION AND TRAJECTORY FORECAST METHODS

Celso S. Barrientos*

The demand for energy and raw materials in the United States continues to increase. Petroleum and its by-products are the main source of energy and materials. This ever-increasing demand has led to a substantial increase in the scale of production and transportation of oil. Subsequently, this led to the increase in the level of pollution by oil, especially in the marine environment where much of the production and transportation are occurring.

Oil pollution in the ocean and the coastal and inland waters is largely a result of natural seepage, continuous seepage during production, loading and unloading operations in port areas, the input of industrial wastes, tanker cleaning discharge in the open sea, and major disasters in marine transportation and offshore production. Disastrous oil spills occur in a more concentrated and spectacular form, often causing severe and noticeable environmental problems. Although disastrous spills have drawn much public attention, the extent of oil pollution in the marine environment is much greater than that caused by the much-publicized major accidents.

The most noticeable damage to the marine environment occurs in the coastal areas. Wildlife and sea life are very susceptible to oil pollution damage. Aesthetic damage to the shorelines is generally severe. The populace is heavily affected by the loss of recreational areas, and the fishing industry and food sources feel the impact.

Although contingency plans to prevent damage by oil pollution have been devised, there is an urgent need to develop methods to forecast movements and the behavior of the pollutant. In the case of oil spills, viable methods are required to predict the oil movement.

The objective of this program is to develop a numerical model to predict the concentrations and trajectories of petroleum in the open ocean (not in bays or estuaries). Concentration will be forecast as a function of space and time. The proposed model will cover various kinds of sources, i.e., continuous point leakage, individual spill, or spill

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added to a general background level of known variable concentrations. The model will include wind, wave, and current forecasts on a small scale in time and space not ordinarily included in the present atmospheric weather forecast system. Forecasting techniques will be developed for these variables. Additionally, trajectory models will require as input oceanic variables, including depth, coastal geometry, and physical properties of the water. These oceanic variables will be used in the development.

Petroleum concentration forecasts by this model will be used primarily by the National Weather Service (NWS) in operational and research modes. The operational mode will be used in real time to augment support of the provisions of the national oil and hazardous substances pollution contingency plan.* In the research mode, potential spills will be evaluated parametrically as in the preparation of environmental baselines to aid decision makers in assessing potential impact of offshore development. A wide range of ultimate users will potentially receive these data. These users include U.S. Coast Guard, Office of Emergency Preparedness, Environmental Protection Agency, U.S. Geological Survey, Bureau of Land Management, and National Marine Fisheries Service.

PROCESSES AFFECTING SPILLED OIL IN THE OCEAN

Four broad classes of processes affect spilled oil: (1) weathering, (2) spreading, (3) diffusion, and (4) transport. These processes are interrelated and interactive. They are acting all the time, but some are more dominant, depending on the time and size of the spill.

Weathering includes processes related to the physical and chemical characteristics of the oil, such as evaporation, coagulation, sedimentation, dissolution, emulsification, and microbial degradation. Some of the weathering processes are dependent on the physical variables of the environment, e.g., evaporation is a function of temperature and wind speed. Weathering processes are ongoing at various stages of the spilled oil; some are more dominant than others at those stages.

Spreading refers to the physical expansion of the lateral size of the spill. Aside from turbulent processes, gravity and surface tension are the main spreading forces. Gravity acts through local oil thickness gradients. Surface tension, which acts on the periphery of the oil, depends upon the chemical and physical properties of the oil and the sea water with which it is in contact. Drag on the bottom of the oil retards expansion. Spreading, which usually occurs simultaneously with diffusion and transport, interacts with these processes.

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* The Department of Commerce, through NOAA, provides support: "...with respect to the marine environmental data; living marine resources; current and predicted meteorological, hydrological and oceanographic conditions for the high seas, coastal and inland waters, including tides and currents for coastal and territorial waters and the Great Lakes." National Oil and Hazardous Substances Pollution Contingency Plan, Council on Environmental Quality, August 13, 1973, 38(155), Part II.

Diffusion is the process by which the size of the spill grows by random motion of the oil and water. Diffusion refers here to turbulent diffusion, which is several orders of magnitude larger than molecular diffusion. Diffusion can be handled in the problem by the use of gradients and eddy diffusion coefficients. There are estimates of diffusion coefficients for spreading of oil, dyes, and other conservative substances in the water. However, the diffusion coefficient for oil may be influenced by spreading as well as by wind fluctuations.

Transport refers to the advective movement of oil as it is being carried by water and wind. When moved by water, we refer to it as motion due to current and when by wind we refer to it as wind drift. Water current is induced by density difference, wind, waves, and tide. Wind drift is the actual action of the wind on the oil. The transport is the vector sum of the currents and wind drift. When oil is spilled, a certain time will be reached when the transport components are probably the most dominant part of the spreading process.

The greatest environmental problem in forecasting oil spill trajectories is the prediction of transport processes. The interaction of the ocean and atmosphere is most important in dealing with this meteorological problem.

MODEL DEVELOPMENT

The general scope of the program is the development of a comprehensive model to predict the dispersion and concentration of contaminants from available oceanographic and meteorological data, including information on the winds from a numerical weather prediction model operated at the National Meteorological Center, NWS; the wave spectrum as determined from numerical models and available observations; bottom topography from available charts; and tidal elevations and currents. The model winds will be used to compute local winds that, in turn, will cause currents and will move floating pollutants directly through drag. Transport from the wave field will be considered as another potential dispersal mechanism. Tidal currents as estimated by the National Ocean Survey tabulations are also important. Bottom topography is needed in several of the computations. A description of bottom topography (and coastlines) must be provided for all coastal regions to be considered. Plans are to concentrate first on the northeast coastal areas of the United States as a case study and, if this approach proves feasible and desirable, to expand the capability nationwide.

This program is interrelated with and depends on a number of ongoing activities in National Oceanographic and Atmospheric Administration (NOAA). Specifically, statistical correlation techniques for estimating local surface winds from model winds have been developed and tested for several coastal locations. Wave spectra are now regularly measured on NOAA data buoys and waves forecast by a numerical model. The wave program will be improved to allow for wave spectra. Bathymetric surveys and their resulting charts are presently a major task of the National Ocean Survey, as are the computations of tidal height and current for most of the U.S. coastline.

The Deepwater Ports Act requires NOAA to make "adjacent state" determinations. The Deepwater Ports Project Office (DPPO) was established in NOAA to perform the functions required by the Act. Trajectory modeling is a primary function of DPPO. Close coordination between this EPA program and DPPO will be maintained. Recently, DPPO has been made part of NOAA's Environmental Data Service.

NOAA's Environmental Research Laboratories (ERL) are involved in environmental assessment of the Alaskan Continental Shelf for the Bureau of Land Management of the Interior Department. A major portion of this project involves ocean circulation and trajectory modeling. This program will apply the relevant results from the ERL's project.

Our program is divided into five tasks: weathering, spreading, diffusion, transport, and model integration.

The first four tasks are the processes group, and the fifth, model integration, includes management, coordination, and implementation of the program. The factors affecting the spilled oil will be treated separately either by contracts or in-house work, and the results will be integrated in an operational model in NWS.

We summarize below the activities involved in each of these tasks.

Weathering

The problems of weathering are complex. Some of the weathering processes are also environmental problems; e.g., evaporation is a meteorological problem. Oil chemists have undertaken a lot of work on weathering; however, this work has occurred mainly in the laboratory, and its application to the actual marine environment is not known. Instead of developing new solutions, we plan to make an extensive literature survey of existing techniques. We will attempt to define the various rates of the weathering processes and include those that are important.

Spreading

The spreading process is dependent on the buoyant and viscous properties of the oil. Where possible, we will apply the pioneering study on spreading by James Fay of Massachusetts Institute of Technology. We believe that there is knowledge of many processes affecting the spilled oil, and interactions with weathering, diffusion, and transport may be significant.

Diffusion

The diffusion process can be handled in two ways; by using classical eddy diffusion coefficients, or by introducing random fluctuations into the equations of motion. To some general degree of accuracy, turbulent coefficients are known for passive tracers, such as neutrally buoyant dyes, but the problem is how this information can be used for oil. The interface between oil and sea water will modify the effects of turbulent water motions on the oil; thus, the coefficients must be altered. Use of the other approach, however, requires a knowledge of the turbulent spectrum of water motions. Its advantage is that both spreading and diffusion can be combined into one equation; thus, interactions can be considered. We plan to investigate both approaches, study the data, and select the more useful method.

Transport

Transport refers to the interaction between oil and water movement. The main problem here is one of coupling: will the oil move as the water moves? Most of our development efforts will be concentrated in this important task. We believe that, in determining oil spill trajectory and concentration, transport will be the major mechanism. We subdivided this task into four subtasks: large-scale or semipermanent current, tidal current, wave-induced current, and wind drift current.

Semipermanent current. We plan to use current tabulations from charts or run large-scale ocean circulation models.

Tidal current. Tidal current is computed or measured for various coastal locations. Tidal current, which is strongly dependent on the location, is significant in the coastal regions and when the time scale is less than the tidal cycle. In the open ocean, it is small and may average out to zero when time is larger than the tidal cycle.

Wave-induced current. Wave prediction models are dependent on the wind input. There are two methods to predict or specify waves: singular method (significant wave height) and spectrum method. There is no agreement yet on a unified theory of wave transport. Simple wave transport solution is that of Stokes. However, surface wave-induced current is still not determined. We plan to do a great deal of development in this subtask, using field and laboratory data and existing models and theories.

Wind drift current. Oil on the water surface will be acted upon by the wind stress. Thus, oil will skid on the water surface because of wind action. When the oil is absent, wind stress on the water surface induces surface current. There are two basic problems involved: (1) prediction of surface wind at suitable time and space scales for oil trajectory; and (2) understanding the mechanism for the transfer of energy from the wind to the oil or water. We plan extensive developments on the specification and prediction of surface wind and studies on boundary layer dynamics. Work on this subtask will be done in-house and by contract.

Model integration. The management part of the project will fall on this task. The program will be performed in the Techniques Development Laboratory of NWS, which will coordinate within NOAA and with other agencies on related studies. This laboratory reviews research proposals and makes recommendations on funding. It monitors contracts and will implement the trajectory model in operational mode in NWS.

ACCOMPLISHMENTS AND STATUS

In this section, we discuss the accomplishments and status of the work for each of the tasks.

Weathering

We have begun a literature survey related to oil weathering in the ocean. Dr. Anand Apte, a National Research Council Post Doctoral Associate, began this survey. We are continuing to do some of the weathering work in-house. For example, we are doing some numerical experiments on evaporation.

Dr. James Mattson of the Center for Environmental Design and Data Analysis, Environmental Data Service, NOAA, is developing a weathering program subroutine for NOAA's work for the Bureau of Land Management. We are following up the progress of Dr. Mattson, and we will incorporate those results that are applicable in our model.

Dr. Ronald Kolpack of the University of Southern California has compiled a two-volume glossary on the fates of oil in the ocean for the petroleum industry. We will be using Dr. Kolpack's suggestions in our model implementation.

We have recommended support of the work of Dr. Christopher Roach on a system model to determine the fate of oil in the marine environment (FOME). Dr. Roach will do the work at Princeton University with Dr. William Gray (see Transport). The FOME model assesses the relative rates of the various processes affecting the spilled oil.

Spreading

In our forecast manual,* we described Fay's experiments and spreading parameters, which are plotted to give the forecasters knowledge of the slick size for an instantaneous oil spill. This approach neglects the effects of weathering and other forces, like wind stress, on the oil. Our experiments are continuing to improve the spreading model.

We developed a numerical model of oil spread based on the radial equations of motion and mass. Although the model was not entirely successful (it did not properly conserve mass), it showed that periodically varying water velocities (such as those that occur in wave trains) have only a minor effect on the rate of spread. For actual conditions, however, a radial model has limited use. We also compared the numerical computations with analytic solutions.

We are programming and testing a generalized two-dimensional spreading model utilizing point-source spills under the action of winds and water currents. We plan to use the *Argo Merchant* spill information to refine the results. One problem will be to include surface tension in the formulation.

Diffusion

Dr. Gabriel Csanady of Woods Hole Oceanographic Institution has a contract on the parameterization of turbulent transfer in the mixed layer. Dr. Csanady, who has completed the scaling analysis for the bottom boundary layer, obtained an elegant formulation to compute effective eddy viscosity. The following two paragraphs are excerpted from the contractor's quarterly progress report:

> "Original data of Lofquist (1960) have been obtained and analyzed in detail with a view to evaluating them from the point of view of modern turbulence theory. This work revealed some unexpected properties of turbulent flow along a density interface, notably a momentum transfer mechanism involving viscous wavelets, which enhance vertical momentum flux associated with the mean shear. The effective viscosity of such an interface has been successfully parameterized. Further analysis, making use of the "inner" and "outer" law framework of turbulent boundary layer theory resulted in the parameterization of interfacial stress. Work is in progress in relating these results to 1972 Lake Huron observations at the bottom of the mixed layer.

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* See Model Integration.

> Somewhat analogous results have been obtained by a reanalysis of data of Wu (1975) on flow underneath the air-sea interface. The effective viscosity was found to be related to surface tension, but the exact method of parameterization remains elusive. Work is in progress on this problem as well as on the free surface velocity law."

We have awarded a contract to Dr. Richard Miksad of the Civil Engineering Department, University of Texas at Austin, on the study of turbulent dispersion motions in both atmosphere and sea. These motions act to diffuse and disperse the slick. The study will deal with air-sea coupling in generating horizontal turbulent spreading and assess its relevance to oil slick spreading. Some simple laboratory experiments will be made to determine the validity of the theoretical results.

Transport

At NWS, we have implemented wind forecast equations for eight coastal stations along the east coast and six along the west coast of the United States. We are monitoring the forecasts and improving the forecast equations. A paper on "Single Station Marine Weather Forecasts Based on Model Output Statistics" by David M. Feit was presented and was published in the Preprint Volume: Conference on Coastal Meteorology sponsored by the American Meteorological Society. This paper describes the development procedure, implementation, and verification of the operational wind forecast techniques for the 14 coastal stations. We have begun developing wind forecast equations for 40 additional stations along the east, gulf, and west coasts, and the Alaskan region.

We awarded a contract to the Institute of Marine and Atmospheric Sciences, City University of New York (CUNY), on "Specification and Prediction of the Vector Wind Stress and Wind Drift on the United States Continental Shelf for Application to an Oil Slick Trajectory Forecast Program." Dr. Vincent Cardone is the principal investigator. CUNY will develop techniques to specify, forecast, and update the driving force for the dominant transport mechanism in an oil slick trajectory forecast model, i.e., the vector wind stress at the air-sea interface. The techniques developed will be oriented to operational applications.

We awarded a contract to the Atmospheric Science Group, University of Texas, on "Oil Spill Behavior in the Open Ocean." The contract is divided into three parts: (1) large-scale atmospheric motion field (Professor K.H. Jehn); (2) atmospheric boundary layer characteristics (Dr. N.K. Wagner); and (3) turbulent dispersive motions in both atmosphere and sea (Dr. R.W. Miksad, see Task 3).

Professor Jehn will determine offshore winds from the more dense observations at the coastal areas by developing a weather map typing procedure. Dr. Wagner will develop a method to transfer the low-level wind field to the ocean surface through boundary layer dynamics.

We have recommended funding the proposed research by R.M. Parsons Laboratory for Water Resources and Hydrodynamics, Massachusetts Institute of Technology, on "Development of a Weighted Residual Scheme to Calculate the 3-D Structure of Currents in Coastal Areas Using a Depth Varying Eddy Viscosity." Professor Bryan Pearce, the principal investigator, will formulate a Galerkin-based finite-element numerical scheme to calculate the three-dimensional velocity field applicable to coastal areas and inland water bodies with forcings by winds, surface elevation, and Coriolis force. The model will be used to investigate the functional form of the vertical eddy viscosity.

We have recommended support of the proposal from the Department of Civil Engineering, Princeton University, on "The Development of a Computer Model for the Simulation of Flow and Oil Slick Movement in the Coastal Zone" with Dr. William Gray as the principal investigator. Dr. Gray will be assisted by Dr. Christopher Roach (see Task 1). Princeton University is proposing to (1) develop a finite element method of solution to the complete hydrodynamic equations for the flow of oil; (2) develop a computer program to determine the fate of oil in the marine environment (FOME, Task 1); and (3) couple the hydrodynamic flow model with the FOME model to arrive at a computer model for ocean oil spill. The proposed model could be used as the core program for the model integration task of the project.

We have recommended funding the proposal from College of Engineering, Rutgers, the State University of New Jersey, on "Numerical Evaluation of the Effect of Ocean Surface Waves on Oil Spill." Dr. Yung Y. Chao, associate research professor, would be the principal investigator. The proposed work consists of three steps: (1) formulation of relevant equations on ocean surface waves; (2) calculation of Stokes velocities for various input conditions; and (3) development of a numerical method to calculate the variation of wave drift velocity at the surface caused by viscous damping of oil film. The result of this study will give us estimates of wave-induced transport.

Model Integration

We completed the first draft of a forecasting manual for oil spill trajectories. The manual includes discussions of the four major processes affecting spilled oil and practical forecasting techniques. The draft was reviewed by the NWS Western Region Headquarters with favorable comments. It was also reviewed by personnel in NOAA's Environmental Monitoring and Predictions Office, who made suggestions for improving the manual. We are revising the manual based on these suggestions.

We are cooperating with other scientists working on the New York Bight data and developing a meteorological analysis technique to derive the forcings for oceanographic models. This input is needed for the oil spill trajectory model. In addition, we are processing meteorological data from December 15, 1976, in connection with the hindcasting for the *Argo Merchant* tanker oil spill accident. The data will be used to test the model.

SUMMARY

We have indicated some of the reasons for the development of ocean oil spill concentration and trajectory forecast methods. Exhibit 1 illustrates the elements in development of an operational ocean oil spill trajectory model and summarizes the important factors determining the fate of oil in the ocean.* There are four main components of the development: software development, and the three main subject areas of weathering, diffusion, and transport. The spreading algorithm is combined with diffusion because of the close interaction between the two processes. We will implement diffusion by solving the dynamical equations of two-dimensional oil spreading, using the turbulent nature of wind and water currents to define or modify the apparent diffusion. Weathering and transport are to be implicitly included in the expansion of the spill.

Total transport will be the combined output of the various water current models (with interaction when appropriate). Initially, values for some of these inputs are expected to be from simplified approximations (such as a wind-driven current proportional to wind speed), but as our knowledge and model development advance, these will be improved. Wave transport will also influence the spreading of the spill. An ice model is necessary in the Alaskan area.

Software development will address and follow the physical and model studies and data gathering, and will dominate the final phase of model integration.

Weather will modify local oil properties (e.g., density, composition, state) to indicate limiting times and areas of contaminants. Winds, temperature, and other atmospheric properties from NWS models will be readily adaptable.

A major part of our progress to date has been in the spreading processes. Our major future task is analyses of transport, which will center on wind-drift currents. We have NWS input of coastal winds and are sponsoring work on air-sea interaction and turbulent transports in the surface layer. All these complementary "on-line" efforts are subservient to the final goal of implementing a forecast procedure. The forecast model will be continually updated as our knowledge and abilities increase.

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* The diagram is based on the one originated by Dr. Jerry Galt of Pacific Marine Environmental Laboratory, NOAA.

EXHIBIT 1: DEVELOPMENT OF OPERATIONAL OCEAN OIL SPILL TRAJECTORY MODEL

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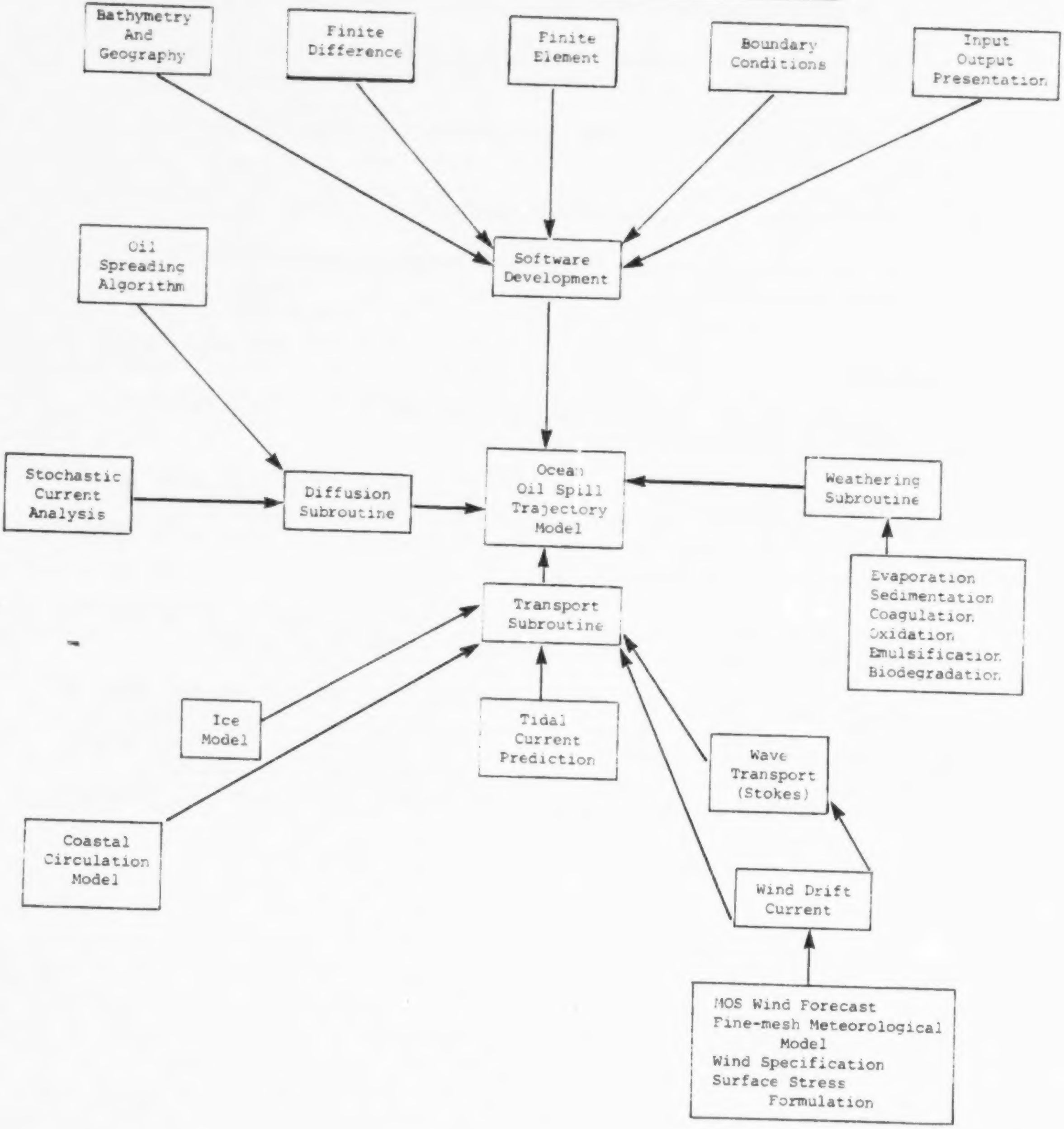

We are also developing the following subprograms:

- A weathering subroutine with the quantitative rates of the processes as a function of environmental parameters

- A spreading subroutine, or a combined spreading-diffusion subroutine, depending on results of our numerical experiments

- A regional meteorological model for analysis and prediction of winds and stability conditions in the boundary layer

- A coastal circulation model to compute currents, including wind drift

- An algorithm to compute transport from wave data

- An integrated oil spill trajectory model including all the above subroutines.

DISCUSSION

From the floor: Do you have any responsibility for analyzing specific spills when they occur?

Dr. Barrientos: It is not clear right now. The last time, in New England, we supported the Coast Guard with meteorological observations. Once this model is developed, we hope to give them more than the meteorological probabilities.

Dr. Baumgartner: Do you intend to provide a service that someone can call when a spill occurs to find out where it is going?

Dr. Barrientos: Yes. Ultimately, that is what we want. That is why we will be needing all the geography in all the coastal areas of the United States and Alaska, so that, at any time, we can just crank up the model for the specific area. The model will be available on the NOAA computer and will have remote terminals all over the country.

THE UNDERWAY WATER SAMPLING SYSTEM

C.D. Kearse,* R. New,† and D. Enabnit*

Many of the open ocean phenomena that have been the subject of oceanographic studies in the past can be characterized by a few data points widely separated in space and time. For example, a salinity change of a few parts per thousand over hundreds of kilometers of open ocean would be considered large, and the important time scales for mixing are several months or more.

With the rise in importance over the last decade of environmental management, the study of coastal and estuarine waters has increased. In contrast to the deep ocean, the spatial and temporal scales are small in the shallow water areas. In an estuary, salinity can change from the marine value of 35 parts per thousand to the fresh water value of 0 parts per thousand in 10 km, and significant mixing caused by the semidiurnal tides can take place in 12 hr. In addition, many of the particular phenomena presenting problems in environmental management, such as pollution events, have temporal and spatial scales significantly smaller than ocean phenomena.

Traditional sampling methods and instrumentation are often ineffective in investigating adequately this new family of smaller scale, more transient phenomena. Because pollution events like oil spills and barge dumps are of short duration, there is insufficient time to stop, collect a sample, characterize the water mass, and move on before the phenomenon has changed significantly. In addition, there may be a net advection of a pollution plume during sampling, which makes it difficult to execute a predetermined sampling strategy. Because pollutant gradients can be large and plumes small, more accurately located and closely spaced sample points are required for adequate characterization. The increase in the total amount of data to be collected can saturate existing instrument systems and become expensive to collect.

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† Now with the Systems Analysis Division, Office of Marine Technology.

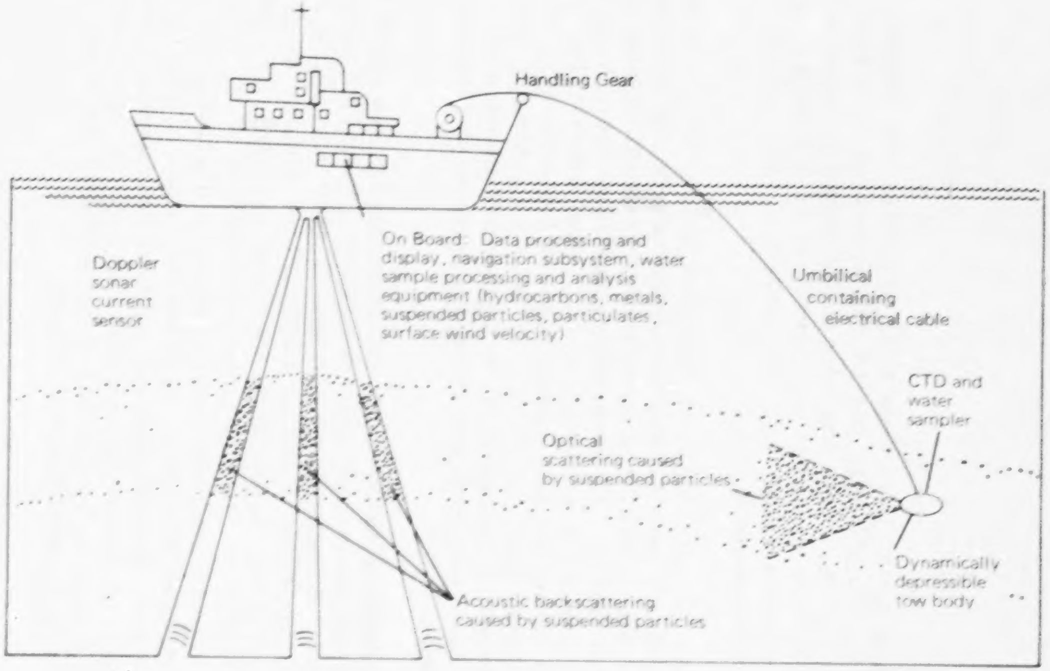
Handling Gear
On Board: Data processing and display, navigation subsystem, water sample processing and analysis equipment (hydrocarbons, metals, suspended particles, particulates, surface wind velocity)
Doppler sonar current sensor
Umbilical containing electrical cable
CTD and water sampler
Optical scattering caused by suspended particles
Dynamically depressible tow body
Acoustic backscattering caused by suspended particles

EXHIBIT 1: A CONCEPTUAL UNDERWAY WATER SAMPLING SYSTEM

Recognizing that the developed technology was seriously lagging, the Engineering Development Laboratory of the National Ocean Survey, National Oceanographic and Atmospheric Administration (NOAA), proposed developing an underway water sampling system (UWSS) to aid in characterizing and monitoring the regions subjected to energy-related pollution. This UWSS is a complete shipboard instrument system designed to acquire, measure, display, and store oceanographic, meteorological, and navigation data while the ship is continuously underway. The Engineering Development Laboratory plans to design, develop, fabricate an engineering model, test, and develop techniques for the use of UWSS.

The system has the following major components: a water measurement subsystem (a towed sensor array or instrumented undulating fish plus a water pumping system) designed to collect oceanographic data and water samples; a navigation, data processing, and display subsystem for navigation, data handling, real-time processing, and display; and a current/depth measurement subsystem to remotely measure ocean currents. Exhibit 1 illustrates this system.

This paper summarizes the efforts to date, describes the present status of individual tasks, and discusses the future of the project. The five tasks that are in progress or completed are:

1. Applications study
2. Navigation, data processing, and display subsystem
3. Current/depth measurement subsystem
4. Overview of optical measurement techniques in oceanography
5. Shipboard chemical measurement techniques.

APPLICATIONS STUDY

The specification of which parameters to measure, their ranges of values, precisions and accuracies, mean time between failure, sampling rate, etc., should be derived from the application in which the system will be used. But the UWSS is an anticipatory system designed to be ready to use when a targeted program begins and not 3 years later. Unfortunately, the NOAA program for the monitoring and characterization of energy-related pollution in which the UWSS will be used is still in its very early stages of formulation, making it impossible to designate the ultimate application. To circumvent this problem, an applications study was performed according to accepted methods of systems analysis. This study was designed to:

- Devise and describe in detail a number of applications of a UWSS that are pertinent to an understanding of the effects of energy-related pollutants in the marine environment.

- Determine whether a "defensible rationale" exists for a UWSS.
    - If the finding is affirmative, then identify what parameters should be sampled and the detailed requirements for sampling.
    - If the finding is negative, then recommend either the speedy termination of the project or the extent and details of a follow-on program.

The study's approach was to have detailed "scenarios" written by oceanographic and environmental experts describing a baseline, monitoring, or special study effort pertaining to energy-related pollution. Each of the eleven scenarios (see Exhibit 2) used a shipborne UWSS as the primary data collection instrument. The major sections of the scenarios described a geographical site, the pollution problem, a mathematical model or data utilization plan, a detailed experimental plan including justification of stated requirements, and a concluding discussion on the advantages and limitations of using an underway system for that application. Appendix 1 contains summaries of five of these scenarios.* In addition to the scenarios, interviews were conducted with oceanographers currently using first-generation underway systems, and their experiences were incorporated into the report.

By selecting a representative set of pollution problems for the scenarios, we produced sample applications that should be similar to those that will be required by the emerging NOAA program. As a result of analyzing these scenarios, we have drawn the following major conclusions:

- There are a number of potentially significant applications in the study of energy-related pollution in which a UWSS would be extremely useful if not essential.
- Underway data collection opens a new regime of spatial and temporal scales to investigation.
- An elementary UWSS that would be useful in many applications can be defined from the common requirements of the scenarios (position, time, salinity, temperature, depth, current, real-time data analysis and display, and the collection of a clean water sample).
- For routine baseline and monitoring programs, there are potential cost savings as a result of the reduced time required for data collection and the automation of sample and data handling.
- Certain sensors and instruments (particularly chemical) may require further development before they will be usable at the higher sampling rates of a UWSS.

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* Complete scenarios are available on request.

EXHIBIT 2: SCENARIOS OF ENERGY-RELATED POLLUTION

Dr. Hosein Fallah & Dr. Robert Stark, Mathematics, and College of Marine Studies, University of Delaware, respectively.
Oil Spill Trajectory: Follow an oil spill to determine the relationship among wind, current, and spill trajectory.

Dr. Keith Stolzenbach & Dr. Donald Harleman: Department of Civil Engineering, Massachusetts Institute of Technology.
Thermal Plumes: Synoptically map a power plant thermal plume in three dimensions.

Dr. Erik Mollo-Christensen: Department of Meteorology, Massachusetts Institute of Technology.
Space and Time Scales: Use underway measurements together with fixed data stations to establish scales of currents.

Dr. James Brooks & Dr. William Sacket: Texas A&M University.
Dissolved Hydrocarbon Sniffing: Baseline determination of dissolved C_1-C_4 hydrocarbons near oil rigs and of relationship of surface slick to petroleum hydrocarbons in the water column.

Dr. James Anderson: University of Washington.
Phytoplankton Patchiness: Distribution and productivity of phytoplankton in pollution-impacted areas.

Dr. Dennis Polis & Dr. Victor Klemas: College of Marine Studies, University of Delaware.
Density Fronts: Study density fronts and provide sea-truth for satellites doing same.

Dr. Douglas Segar: NOAA/EDL.
Trace Metals: Perform baseline assessment of trace metals in New York Bight and dedicate ship for monitoring pollutants at dispersed locations.

Mr. Earl Merritt: Earth Satellite Corporation.
Satellite Sea-Truth: Use UWSS to provide sea-truth for satellite data of Pacific updwellings.

Dr. Roff McCluney: Florida Institute of Technology.
Optical Properties: Monitor optical properties in marine areas subject to dredging.

Dr. James Mattson: NOAA/Center for Experimental Design and Data Analysis (reduced effort).
Oil Spill: Mapping surface and subsurface components of NOAA test spill in Alaska.

Dr. Alan Blumberg: NOAA/GFDL (reduced effort).
Numerical Models: Provide boundary values for a numerical model of New York Bight.

This applications study is essentially complete and is being prepared for publication. The next step is to take the study to NOAA operational program managers to determine how a UWSS can be exploited in present and future programs. The output of this step will be considered judgment as to the specific and most valuable application(s) for underway systems in NOAA. From these applications, the Engineering Development Laboratory can detail the specific parameters to measure and, using the additional information in the scenarios, determine a set of functional specifications and proceed with the development. Because the ultimate usefulness of the UWSS is critically dependent on designing for the correct application, it is essential to estimate as closely as possible the ultimate application. For this reason, the applications study is the most heavily emphasized aspect of the UWSS project at present.

NAVIGATION, DATA PROCESSING, AND DISPLAY SUBSYSTEM

Because some of the major subsystems will require long development time, it is necessary to begin their development before final definition of the total system. The danger in this procedure is that these subsystems may not be required in the ultimate application. However, in our best judgment, the navigation, data processing, and display subsystem and the current/depth measurement subsystem will be needed in any system, so work began on them before the applications study was completed.

The navigation, data processing, and display subsystem (see Exhibit 3) provides such functions as data logging, display, real-time data processing, and control of sensor operation and sampling rates. It also will provide accurate vessel navigation, utilizing input from present navigation devices, such as LORAN, OMEGA, and RAYDIST. The output will be raw data on magnetic tape, a summary listing of selected raw data, a hard copy real-time navigation plot of data acquisition tracklines, and selected processed data displayed in real time. The whole UWSS is to be interactively controllable by the scientist through this subsystem.

The system hardware is based on a PDP 11/40 and includes the PDP 11/40, an A/D, D/A converter, a digiview display, tape drive, disk drive, and a keyboard/printer. The Engineering Development Laboratory has purchased, installed, tested, and modified these items, which are presently operational. Funds from the Environmental Protection Agency (EPA) have been used to augment the computer with a plotter and floating point hardware.

Software is also being developed. Because the application for the UWSS has not been designated, the software is written in basic modules that can be assembled later to form the actual programs. Two categories

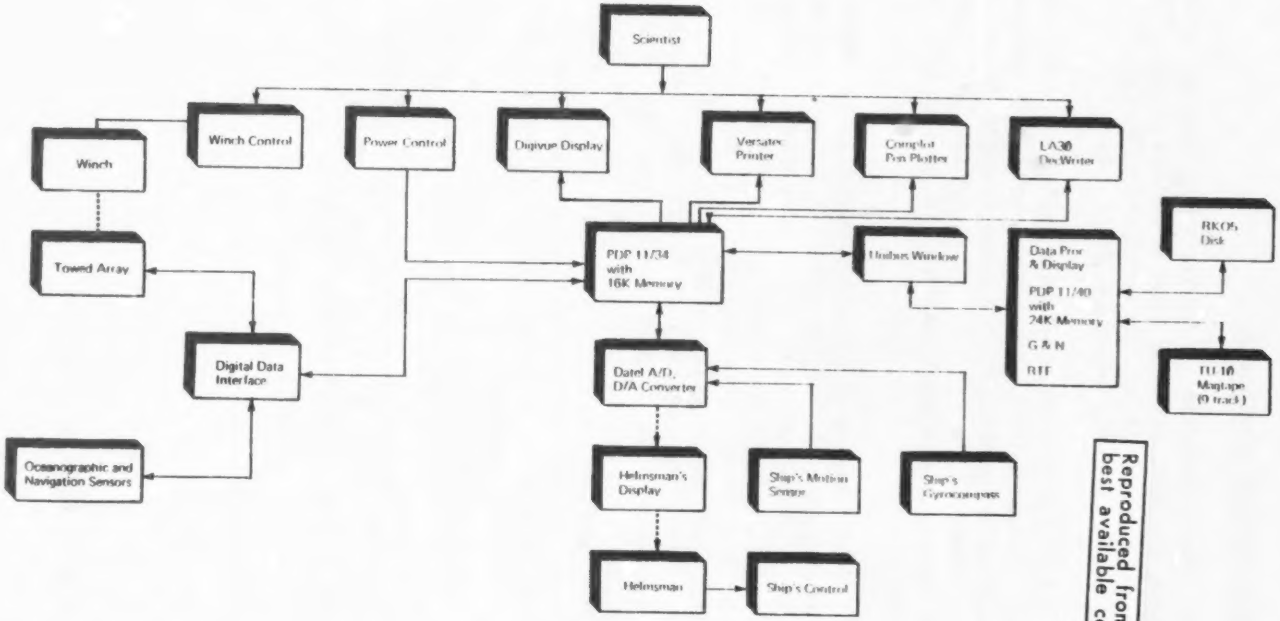

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EXHIBIT 3: UWSS FUNCTIONAL DIAGRAM OF HARDWARE CONFIGURATION

of modules have been developed and documented to date:* a systems macro library of links, prints, and special functions for use with the navigation software and in doing general computations; and a set of navigation coordinate systems conversion modules to make conversions such as X-Y geographic positions and range-range to X-Y. A large amount of software development still remains to be performed.

CURRENT/DEPTH MEASUREMENT SUBSYSTEM

The goal of this subsystem is to remotely measure a vertical profile of subsurface current vectors from a moving ship. This subsystem will operate either as a part of the shipborne UWSS or in a stand-alone mode, a difficult but highly desirable goal.

After the potentially applicable technologies for remote current measurements were investigated within Engineering Development Laboratory, a series of functional specifications were written for a current/depth measurement subsystem, and a request for bids issued. After completing the procurement cycle, a contract was signed to develop, fabricate, and test the subsystem.

The current/depth measurement subsystem will be based on the principle of acoustic Doppler shifts to remotely measure water currents. An acoustic pulse is transmitted from a hull-mounted transducer, and both volume reverberation (echoes from acoustic scatterers suspended in the water) and the bottom return are detected. If the scatterers are moving with the current, then the Doppler shift of the volume reverberation gives the speed of the scatterers and, hence, the current. The bottom return is used to remove the Doppler shift from the ship's motion and to measure water depth. By emitting three beams in different directions, the current vector can be resolved. Using a range-gating technique on the return signals, a vertical profile of currents can be measured. The analysis and interpretation of these returned signals present a significant and controversial problem.

The contract for this subsystem is in three phases:

1. Theoretical analysis of proposed system, production of a total system error model, design modifications on a similar system developed for the French Navy, and analysis of data from a test of the French Navy system
2. Hardware fabrication
3. Test and evaluation.

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* Copies are available on request.

At preestablished points, progress is evaluated with continuation of the contract dependent on satisfactory completion of the preceding work. Presently, the contract is in Phase 1 with the French Navy test still to take place.

With its own funds, the Engineering Development Laboratory is sponsoring an in-house research program on the Doppler acoustic technique to further the probability of success on the current/depth measurement subsystem.

OVERVIEW OF OPTICAL MEASUREMENT TECHNIQUES IN OCEANOGRAPHY

Early in the UWSS project, there seemed to be a high probability that at least one optical sensor would be required as a part of any system. To build a base of knowledge of relevant instrumentation, we performed a study of state-of-the-art measurement techniques in optical oceanography. This information has been incorporated in a report and is available for use when the application is designated. The report itself is undergoing peer review in the academic community prior to release.

SHIPBOARD CHEMICAL MEASUREMENT TECHNIQUES

There is a high probability that any UWSS will incorporate chemical instrumentation. The Engineering Development Laboratory performed a survey of presently used techniques of shipboard chemical analysis. This information will define developments in analytical instrumentation that must be made before incorporation in a UWSS.

FUTURE OF THE PROGRAM

The immediate future of the UWSS is not bright. EPA has decided not to continue funding the project. This is unfortunate since we and the Office of Ocean Engineering (the NOAA overview office for this project) are convinced that the concept is of considerable merit. Interest in the UWSS is also increasing elsewhere within NOAA. Both Marine Ecosystems Analysis and the National Ocean Survey Ocean Dumping Program, major operational programs of NOAA, are interested in trying a UWSS in their applications. Representatives from NESS, Center for Experimental Design and Data Analysis, National Environmental Satellite Service, and the Geophysical Fluid Dynamics Laboratory have been involved for some time in discussions with the Engineering Development Laboratory on how a UWSS could be used in their applications and what specific requirements they would have.

To obtain the full value of the work that has already been done, Engineering Development Laboratory intends to continue the applications study to its logical conclusion, i.e., a decision by NOAA management as to the specific and most valuable NOAA application of a UWSS. This will involve finishing the study, meeting with major program managers and planners, and a formal decision.

The premature end of this program is disappointing. Only rarely is it recognized that scientific programs have a responsibility to continually help advance the state of the art in instrumentation and instrument systems. Yet if this responsibility is not met, new and more difficult problems will be met with progressively more archaic instruments.

APPENDIX 1: SUMMARIES OF FIVE SCENARIOS

We summarize below five scenarios: trace metals, dredge spoils, biological effects, thermal plumes, and hydrocarbon sniffing.

Trace Metals

Many chemical contaminants are introduced to the coastal marine ecosystem as a result of the energy-related activities of man. The impact of these contaminants on the biosphere is largely unknown because of the paucity of information concerning the chemical ecology of coastal ecosystems. In his scenario, Dr. Segar uses an UWSS, with onboard analysis, to perform a chemical oceanographic baseline survey of the New York Bight Apex. The apex is a region presently and potentially affected by man's activities related to energy production, transportation, and utilization.

A baseline survey of the bight was performed as part of NOAA's Marine Ecosystems Analysis Program. The program was a landmark in chemical oceanography, but like all first-of-a-kind studies, posed as many new questions as it answered. The scenario describes how the initial baseline assessment of New York Bight was done, develops a strategy to perform this assessment with an underway sampling system, and demonstrates how the use of such a system would render the assessment of much greater value.

The proposed, improved baseline survey of New York Bight Apex consists of a minimum of three, 5-day cruises over a 1-year period with standard trackline surveys taken over the same sampling grid on the first, third, and fifth days. The remaining 2 days of each survey are reserved for detailed study of the areal extent and time history of anomalous contaminant plumes observed during the standard distributions of chemical parameters in the study area. These distributions need to be detailed (horizontal changes in concentration must be resolved over distances of $\leq$ 1 mile) and to be obtained within a short period ($\leq$ 24 hr) to enable the resolution of the temporal changes of several days that are characteristic of the New York Bight Apex.

In a second task, the onboard analysis, the underway sampling system is used to perform the continuing monitoring required in the New York Bight Apex. This is achieved by a single repetition of the baseline survey cruises several times each year. This monitoring strategy would identify long-term changes in the ecosystem and frequent short-term events, but would require supplementing with source monitoring to identify isolated short-term contamination.

The chemical parameters that would be determined onboard would vary for each affected coastal region. The parameters could be changed as more knowledge of the ecosystem is gained. Since some of these chemicals cannot presently be analyzed rapidly aboard ship, the underway sampling system must prepare and store samples for later laboratory analysis. A detailed list of the parameters is collated in the scenario, which is too extensive to include here.

Using a UWSS for the baseline survey and monitoring has several advantages over conventional station grid surveys with shore-based analysis:

1. The system provides the most cost-effective, least labor-intensive, and least contamination-prone means of fully utilizing the capabilities of the available analytical instrumentation. Instrumented analysis systems for a number of parameters are now available that are capable of either analyzing a continuous sample stream or accepting small volume samples for analysis at a rapid rate. Obtaining water by standard hydrographic sampling and supplying discrete samples to these instruments at a sufficient rate to utilize fully their capabilities are virtually impossible and inferior to a continuous sample stream.

2. The temporal and/or spatial resolution of chemical concentration variations will be improved if the pumped sample stream is obtained with the vessel underway and the rapid analysis capability utilized. The underway system eliminates station time and, therefore, more locations can be sampled in a given time. Characterization of the chemical distribution in a given area can be completed more quickly and repeated more often.

3. Events of short duration that are subject to water mass motions may be followed using the underway sampling capability and the interactive capability of onboard data display. The interactive capability provided by the onboard analysis and data display is essential to optimize sampling of a dynamic anomaly.

Dredge Spoils

Energy-related pollutants, such as biocides from power plant heat exchanger cleaning operations, heavy metals from shore-based energy-related manufacturing processes, and petroleum products have been found to accumulate in bottom sediments. Short-term resuspension of several layers of this polluted sediment can result in toxic concentrations of these materials in the water column. Through these mechanisms, dredging operations can have a strong impact on the introduction and distribution of energy-related pollutants in coastal areas. In his scenario, Dr. McCluney uses a UWSS to determine the generation and transport of particulates caused by dredging in Tampa Bay, Florida.

There are additional effects of increased suspended sediment concentration. Organisms such as benthic algae, other bottom vegetation, and some marine animals are sensitive to increases in turbidity. High turbidity blocks out sunlight and reduces photosynthesis in bottom vegetation and near-bottom phytoplankton. The siltation accompanying dredging may destroy rooted bottom vegetation, exposing bottom sediments to wave and tidal action that can further increase the turbidity and spread the effects of siltation. The marine ship-channel dredging operation planned for Tampa Bay threatens continued water quality degradation, unless the mechanisms of particulate transport and resedimentation can be determined and the information used to minimize the adverse consequences of the project.

Dr. McCluney will use the underway system to perform a detailed particulate concentration survey of a dredge spoil plume. The resulting information will be used to develop a better understanding of the mechanisms of particulate transport and deposition related to dredging operations in Tampa Bay.

The strategy will be to criss-cross the dredge plume with a series of roughly parallel transects to monitor continuously the particulate concentration in each size range at reselected depths. In addition, the wind, bottom depth, current, and salinity will be measured. A complete series of 40 transects across the plume will be completed in 1/10 the semidiurnal tidal period of 10 hr to provide adequate resolution. A series of five consecutive surveys will be conducted over one full tidal period. These surveys will be repeated under varying conditions of wind strength, wind direction, and fresh water runoff.

Then, a UWSS will be used to determine the areal extent of the effect of dredging on sunlight penetration. This task is designed to take a broader look at the effects of dredging in a large area surrounding the dredge; the emphasis is to be upon developing a relationship between changes in light penetrability and the concentration of dredged particles. The particulate size and index of refraction will be used to obtain an estimate of the ratio of dredged particle concentration to ambient particle concentration. Increases in the ratio from dredging operations will be compared with changes in the penetration of sunlight to the bottom to determine empirically any correlations that may exist between these two parameters.

The underway system will perform a detailed particulate concentration survey of the region surrounding a dredge plume. The principal parameters measured will be the particulate concentration in each of the size ranges and in each of several ranges of particulate index of refraction. The particulate concentrations and irradiances will be measured at each of a series of horizontal locations covering an area of approximately 100 km (more than 10 times the expected area of the visible dredge plume). The salinity, current, wind, position, depth, and time will also be measured.

The strategy will be to criss-cross the dredge plume with a series of roughly parallel transects and to monitor continuously the above parameters. A survey of the study area will be completed in 1/10 the semidiurnal tidal period of 10 hr. As with the preceding task, these surveys will be repeated at five different stages of the semidiurnal tidal cycle, during varying conditions of wind strength, wind direction, and fresh water runoff rate. It is very important that a complete tidal cycle series of these surveys be conducted before the start-up of dredging operations to provide baseline information.

For this scenario, the underway mode of data collection is seen as a technique for providing needed water quality data over large areas with the relatively high spatial and temporal resolutions required, but without greatly increased costs for collecting these data.

Biological Effects

The actual effect of pollution on the environment is a question of major concern. In his scenario, Dr. Anderson uses an underway system to study the impact of energy-related pollution on the lower trophic levels of the marine food chain.

The marine food chain consists of four pools of living organisms and a pool of nutrients and solar energy. Each pool "feeds" on the pool beneath it in the chain. At the lowest level, plants feed on the nutrients and solar energy. Proceeding up the chain, herbivores feed on the plants; carnivores (fish) feed on the herbivores; and man feeds on the carnivores. If pollution adversely affects the plankton and herbivores, the supply of fish available to man can be affected through two mechanisms: the fish themselves pass through these lower trophic levels as planktonic eggs and herbivore larvae; and these two trophic pools serve as a food source for fish. Therefore, the impact of energy-related pollutants on these lower trophic levels is directly linked to the productivity of commercial and sport fisheries.

To identify critical interactions between the marine food chain and energy-related pollutants, the following information must be collected:

- Biomass distributions of the relevant trophic pools

- Body size and life-cycle stage of the dominant species in each trophic pool
- Dominant species composition of each trophic pool
- Rates of biologically mediated fluxes between the trophic levels
- Current field
- Physical fluxes of the trophic pools
- Active transport by sinking and migration of the organism
- Diffusion coefficients when relevant to a trophic pool.

This information is required in the differential equations used to model the biological pools. Dr. Anderson has proposed three tasks, using an underway system to collect the above information.

In the first task, the growth limitation of plankton caused by pollution will be measured, by following an isopleth, such as the chlorophyll maximum, over the entire length of Puget Sound. This must be done in 6 hr and repeated during periods of maximum plankton growth and herbivore grazing. By comparing polluted and unpolluted regions, it should be possible to document the cell size change of plankton and the spatial extent of the change. This task takes advantage of the UWSS's capability of rapidly sampling a large area such as Puget Sound to determine the magnitude of a pollution-biota interaction.

The immediate mortality caused by an actual, accidental oil spill will then be determined in task 2. This task requires that a "ribbon" of data be collected. A data ribbon is a line of continuous data near the surface, a line of continuous data at a fixed depth, and a continuous line of data oscillating between the upper and lower fixed data lines. From ribbons of current data and the plankton concentration, the physical flux can be subtracted from the total flux leaving the biological flux. The biological flux can then be compared to a neighboring unpolluted region to determine the oil-induced mortality (which is a biological flux). The task takes advantage of the UWSS's ability to furnish a continuous sample for analysis.

In task 3, the difference between physically and biologically mediated fluxes of several parameters will be determined. It is the biologically mediated flux that passes energy up the food chain and ultimately determines the fishing flux. This information can be used in the first two tasks to distinguish physical flux effects from pollution-induced growth and mortality. To perform task 3, a selected parcel of water will be continually circumnavigated and bisected. Since the system is underway, the same parcel of water can be measured even though it translates with the current. From the collected data, the vertical and lateral physical fluxes can be computed. A total volume rate of change in the trophic pool parameters can also be computed. The difference between the total rate of change and the biologically mediated rate of change is the physically induced flux.

To perform the above three tasks, an underway data collection system needs to be able to measure: light intensity in the water, chlorophyll fluorescence, millimeter-size particles, temperature, salinity, depth, current, the specific pollutants, nutrients, and dissolved oxygen. It must also be able to: collect and filter a continuous water sample for zooplankton; determine ^{14}C uptake, election transport system activity, and ATP concentration; and identify plankton and herbivore species.

The major advantages furnished by underway data collection are: increased data density, ability to follow a moving parcel of water, and collection of data over a large area in a short time.

Thermal Plumes

One of the energy-related pollutants that has received a large amount of public attention is thermal pollution from power plants. In his scenario, Dr. Stolzenbach uses a UWSS to help analyze and monitor the heated discharge from a large offshore electric generating station.

Of the 10^{16} Btu of fuel used in U.S. steam power plants, approximately two-thirds is rejected into the environment as waste heat. When discharged into the marine environment, this waste heat can alter the dissolved oxygen concentration, change the growth rates and behavior of aquatic organisms, and cause death in the biota from exposure to elevated temperatures. The biological effects are known to depend on the frequency and duration of exposure to elevated water temperature, and the total magnitude of the effects will depend on the amount of water affected. Therefore, to study the impact of waste heat on the environment, it is necessary to know the full temporal and spatial distribution of the induced temperature changes.

One source of marine thermal pollution will be the Atlantic Generating Station (AGS), a proposed 2300 MWe floating nuclear power station to be anchored off Little Egg Inlet, New Jersey. The AGS will use once-through cooling and discharge 4640 cfs of water heated to 16.1°F above the ambient. After mixing with the receiving ocean water, the heated discharge will establish some farfield temperature distribution. This distribution has been modeled as a differential heat conservation equation. The first task proposed by Dr. Stolzenbach is to use a UWSS to measure the site-specific coefficients in the farfield model.

The site-specific coefficients are the temperature diffusion coefficients for all three dimensions. These coefficients will be measured using a dye tracer to simulate the thermal plume. Once the dye has been dispersed, a three-dimensional map of temperature, dye concentration, position, and time must be collected as a series of snapshots. The ability of an underway system to build such a map in a highly synoptic fashion is a new capability with wide applicability in oceanography.

Task 2 involves measuring the actual farfield temperatures resulting from the AGS power plant discharge. The objective is to construct a three-dimensional contour map and to monitor its change with time. Since many of the ecological impacts are a function of the duration of exposure to elevated temperatures and the magnitude of the temperature increase, a time-varying contour map, which allows one to describe how often and for how long different volumes of water are subjected to what elevated temperatures, can delineate the critically affected volumes. To construct such a map, a system towing a string of temperature sensors will sweep back and forth through the plume, recording temperature, position, and time. A single, moored current meter will provide data to remove the effects of tidal current displacement.

Hydrocarbon Sniffing

The production of oil from offshore wells is a source of oil pollution in the sea. In their scenario, Drs. Brooks and Sackett use a UWSS to determine the transport and fate of production-related oil pollution on the Louisiana Shelf. Two specific pollution sources are investigated: underwater venting of the natural gas by-product of production, and accidental spills at a production site.

A small amount of natural gas is recovered as a by-product of oil production. Because it occurs in uneconomically recoverable quantities, the gas is burned or vented underwater. The primary components are the C_1-C_4 light modular weight (LMW) hydrocarbons, but a certain amount of the toxic water-soluble C_5-C_{12} compounds is also vented. The LMW hydrocarbons are valuable short-term traces of these toxic, soluble components of petroleum. A system known as a hydrocarbon "sniffer" is used to measure these hydrocarbons. A "sniffer" is a submerged pump connected by flexible hose to a gas separator, a gas chromatograph, and a total hydrocarbon detector aboard ship. The sniffer is towed at some depth through an area of interest while sampling and analyzing the water for LMW hydrocarbons.

The sniffer system will be used to make a detailed concentration map of the hydrocarbon plume produced by venting. From such a map, one can determine the dissolved hydrocarbon composition, the directions of advection of the surface and subsurface plume, the dilution of the oil, and the loss of dissolved hydrocarbon to the atmosphere. The survey tactic will be to prescribe a 2-mile square around the vent to locate and size the plume and then to sweep back and forth across the plume at continually greater distances from the vent. Water will be pumped from two depths to analyze for LMW hydrocarbons and for total hydrocarbons.

Oceanographic parameters (salinity, temperature, dissolved oxygen, current, and depth) will be collected simultaneously with the water samples.

Two new advantages of an underway system have been demonstrated here. Since station time for sample collecting has been eliminated, one has enough extra time available to run a preliminary 2-mile square around a vent. This allows the user to optimize the rest of the survey by eliminating many measurements outside the plume. A second advantage is that sample collecting has been automated and, therefore, is much easier than the traditional hydrocast.

The underway sniffer will then be used as a quick reaction system to study the dissolved components in an accidental oil spill. For example, assume there has been a platform mishap resulting in an accidental release of oil to the sea. The underway sniffer could be deployed quickly to examine the spatial distribution and depths of penetration of LMW hydrocarbons as they dissolve and evaporate. Because the light hydrocarbons C_1-C_{12} evaporate quickly, about 50% of the spill will be lost to the atmosphere in a few hours. Thus, the ability of the underway sniffer to survey an area rapidly is essential.

The same data are required in both tasks, and both require an interactive capability. The underway system must be able to take samples, analyze them, and display the results in real time so an experimenter can tailor the survey to the actual oil plume.

This scenario describes an underway system that is already in operation and that the authors have used. Therefore, advantages that Drs. Brooks and Sackett claim for the technique are proven advantages, i.e.:

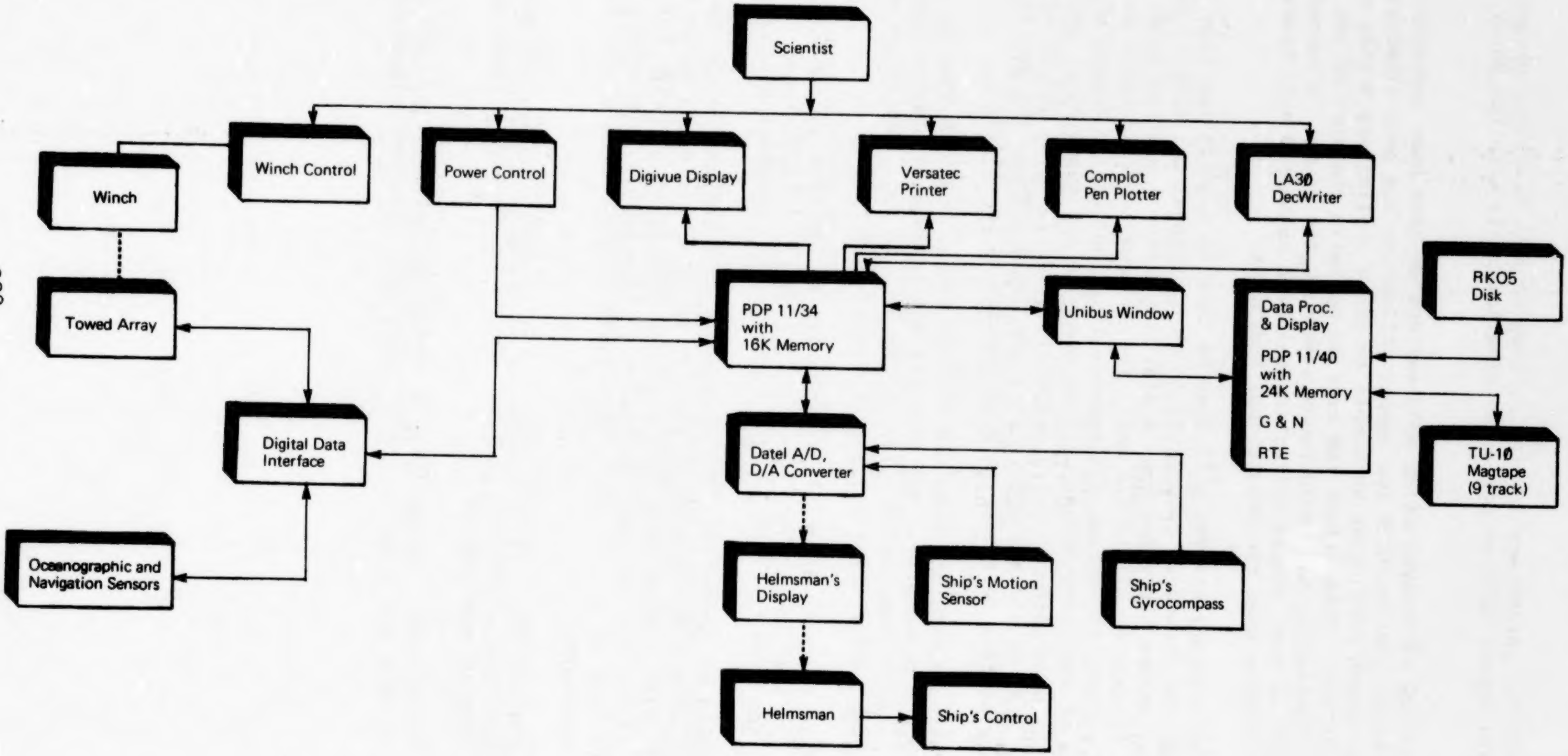
- Speed of operation. The system supplies samples to the analytical equipment at the maximum rate at which analysis can be performed. It also travels from data point to data point quickly because station time has been eliminated. As a result, the sniffer can perform in 2 hr a survey that would take 24 hr by conventional techniques.

- Adaptability. Coupled with real-time data display, the speed of operation allows one to alter the survey tactic in response to a changing environment.

- Convenience. By supplying a continuous water stream, the inconvenience and labor of taking hydrocasts have been eliminated.

Exhibit 2

UWSS Functional Diagram of Hardware Configuration



SHIPBOARD ENVIRONMENTAL DATA ACQUISITION SYSTEM

Douglas W. Downen and Richard R. Reynolds*

Since 1853, the National Weather Service (NWS) has operated a volunteer observing program aboard ships to provide support to marine interests in the form of forecasts and warnings. These forecasts and warnings must be kept updated at all times.

Observations from data-sparse ocean areas are very important to good forecasts as well as timely warnings of potential problems. With increasing emphasis on utilization of the oceans as a source of food and energy, the large user community for environmental marine data is growing. In addition, this increased use of the oceans has brought about a new concern about ocean dumping and pollution.

Recently, there has been concern whether NWS receives sufficient observations to support their mission. Observations are collected from moving ships and fixed platforms and transmitted over various communication routes. Presently, these communications are inadequate. Many of the observations never reach the intended destination or arrive there too late to be of value to the real-time user. Only a small percentage of observations made by the ship's crews are actually transmitted and delivered to NWS forecast offices. Of the approximately 60,000 marine weather observations taken each month, only 30,000 ever reach the National Meteorological Center (NMC). Only 9,000 of these are in time to be included in the first map analysis. Furthermore, there are periods when nearly no data are available from certain ocean areas.

Real-time reporting is now feasible, and satellites in geostationary orbits can now provide a direct data link from ships to NWS. Currently, the NWS is developing an automated environmental data observation system for shipboard use. Known as the Shipboard Environmental Data Acquisition System (SEAS), this system will provide real-time monitoring of meteorological and oceanographic parameters. It will be operated on board various ships (e.g., oil tankers, merchant ships), and the observed environmental data will be transmitted via satellite communications to NWS in Suitland, Maryland. The SEAS will provide routine

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observations from relatively data-sparse oceanic areas. Correlation of these data with additional environmental data will result in improved weather forecasts and warning services.

The SEAS data and improved environmental data forecasts will be available for a wide range of users (e.g., coastal zone managers) to analyze offshore exploration and resource exploitation, deepwater port operators, and tanker routing consultants. In addition, it will be used as input to the oil spill concentration and trajectory forecast model currently under development by the Techniques Development Laboratory, Systems Development Office, NWS. This model will assist in providing more efficient clean-up operations in the event of a spill.

Many other energy and pollution-related activities will benefit from SEAS. For example, with pollution clean-up operations aided, the possibility of spills reaching coastal areas will be reduced, thus preventing damage to beaches and death to ecosystems. Improved forecasts of heavy seas and strong winds will be used by ship-routing personnel to steer tankers away from dangerous storm areas to avoid tanker breakups. In addition, the efficiency of oil tanker operations will be improved with better environmental forecasts.

The SEAS program is being developed in a series of phases culminating in a fully automated meteorological/oceanographic observing system. The first phase is the engineering model (SEAS-1), which was designed and built under a contract with Industrial Meteorology, Indian Harbour Beach, Florida. Installation of SEAS-1 on the NOAA ship Researcher was completed in August 1976, and since that time it has been undergoing test and evaluation. The primary purpose of SEAS-1 is to demonstrate the practicability of using an automated system for the acquisition and communication of environmental data in a harsh shipboard environment.

Exhibit 1 depicts the basic components of SEAS-1. The wind and temperature sensors are mounted on the mast, the data collection platform radio set (DCPRS) mast electronics are on the stack, the pressure sensor is just forward of the operations control center (OCC), and the sea water temperature and conductivity sensors are in the bow bubble of the Researcher. The central terminal unit housing the display, computer, keyboard, and DCPRS is in the OCC. The keyboard is used by the observer to input ship's position, course and speed, and visual parameters (e.g., clouds, obstructions to vision, visibility).

SEAS-1 transmits a complete marine weather observation at three-hourly intervals, which is transmitted via the Geostationary Operational Environmental Satellite (GOES) to the NWS Wallops Island, Virginia, Receiving Station. It is then transmitted to Suitland, Maryland, for correlation with additional environmental data. In addition, SEAS-1 displays all observed and transmitted data at the central terminal unit.

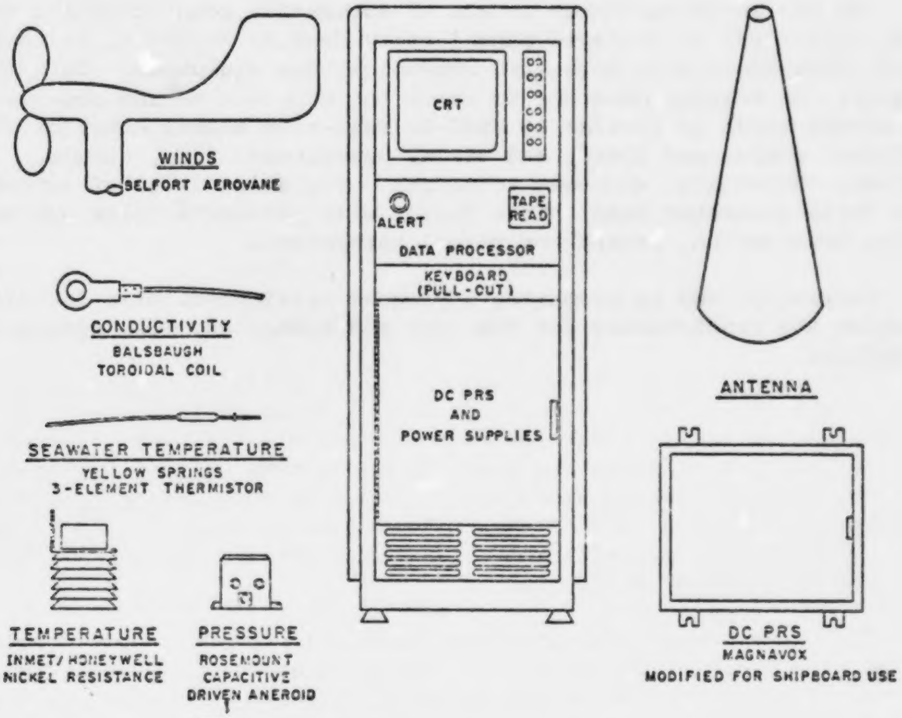

EXHIBIT 1: SEAS-1 CONFIGURATION

The sensors and processing system have functioned without any failures. The only problems encountered to date have been in the satellite communications, and the antenna seems to be the primary problem. A newly designed antenna will be tested this spring.

An automated expendable bathythermograph (XBT) launch system for SEAS is being developed by Plessey Environmental Systems, San Diego, California. This system will be integrated with SEAS-1 in March 1977 and undergo operational test and evaluation on the Researcher. The XBT system has the capability of automatically launching XBT probes from ships cruising at speeds up to 30 kt and providing data from depths up to 760 m. The launch portion of the system is capable of storing a ready supply of 48 probes. The need for ship personnel assistance will be minimized with this storage capability. Data measured by the XBT system will be processed by the SEAS-1 data processor, and a standard bathythermograph report of sea water temperature versus depth will be transmitted.

To gain experience with typical ship operations personnel, we plan to install SEAS on a ship-of-opportunity this fall. Since many merchant vessels are now using the Maritime Satellite (MARISAT), we may reconfigure SEAS-1 to use this communications medium in lieu of GOES. However, economic considerations lean toward using GOES.

The NWS envisions three levels of automation sophistication for SEAS. The first is a simple manual entry device, keyboard, or thumbwheel, interfaced with satellite communications equipment. This system requires the weather observer to input the complete marine observation. The second level is similar to SEAS-1, requiring manual entry of ship's position, course and speed, and visual parameters, i.e., clouds, weather, visibility, and wave formation. The third level of automation is a fully automated SEAS. This design will provide a fully automated marine observation, except for visual parameters.

Currently, NWS is preparing a program development plan for SEAS, in which the requirements for the type and number and SEAS systems will be defined.

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